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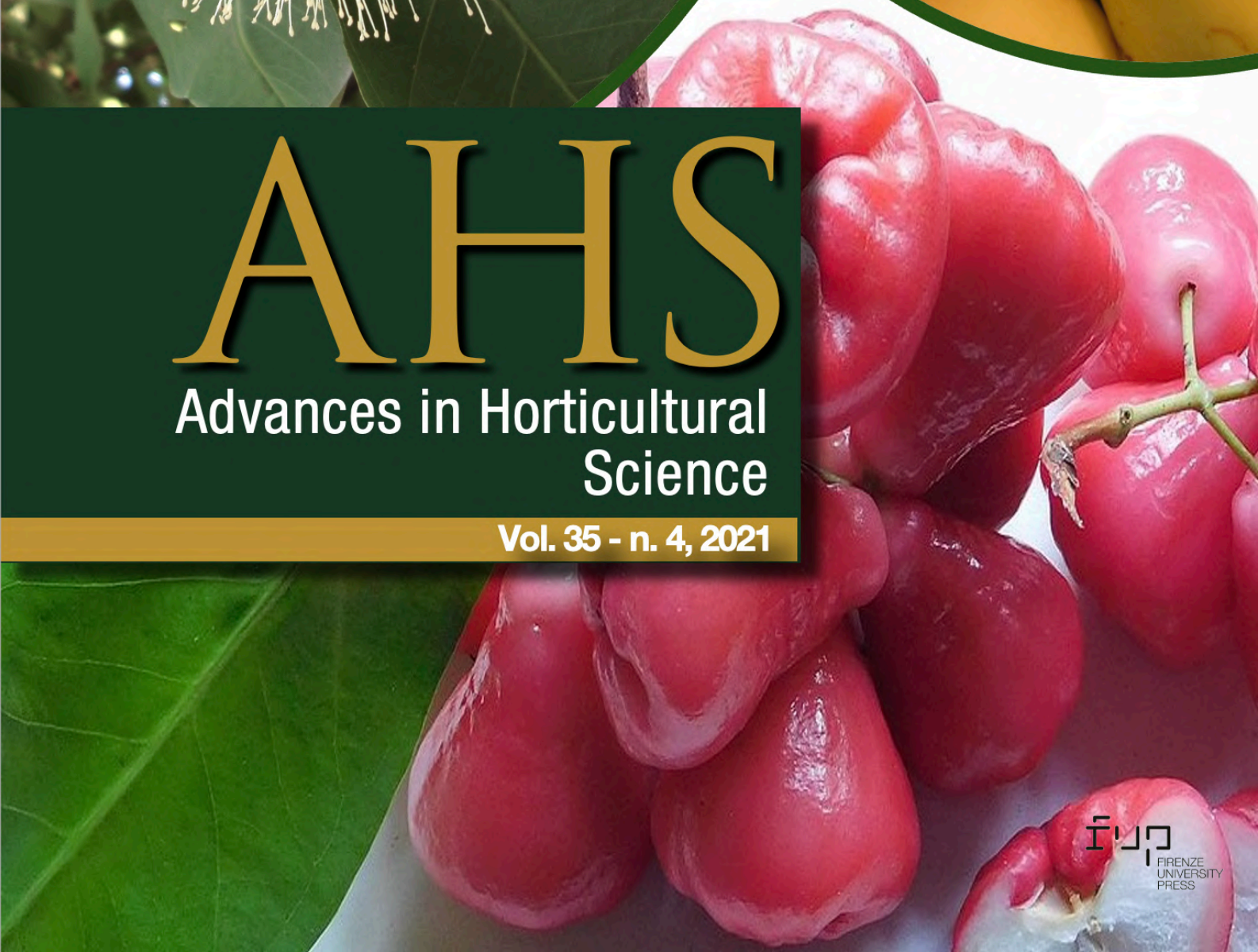

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Exogenous salicylic acid and ferulic acid improve growth, phenolic and carotenoid content in tomato

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Abstract: Salicylic acid (SA) and ferulic acid (FA) are considered phenolic compounds that act as elicitors due to their regulatory functions on plant growth, development, metabolic and physiological responses in plants. The aim of this research was to evaluate the effect of SA and FA on growth, fruit quality and synthesis of secondary metabolites in tomato (*Solanum lycopersicum* cultivar Santa Clara). The experiment was conducted in pots in a greenhouse. The application of SA and FA was performed at concentration of 1.0 mmol L^{-1} alone and in combination, with water treated plants as control. Exogenous application of SA and FA either alone or in combination (SA + FA) resulted in increases in biomass accumulation and chlorophyll contents in tomato plant; and soluble sugar, total polyphenol, flavonoids, lycopene and β-carotene contents in fruits. It was concluded that application of SA and FA resulted in higher production and concentration of secondary compounds in tomato.

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and it is considered as low-calorie food. It is a good source of vitamins A, C and E, and mineral salts (Adalid *et al.*, 2010; Kazemi, 2014). In addition to vitamins and minerals, tomato has a high lycopene and β-carotene content, which has antioxidant and anticancer properties. Carotenoids is naturally present in many fruits and vegetables and plays an important role as a functional food when consumed as part of the diet, producing specific health benefits such as reducing the risk of various diseases (Martínez-Hernández *et al.*, 2016; Mehta *et al.*, 2018) and polyphenols (flavonoids, flavanones and flavones) are also present in significant amount in tomato acting as antioxidant, anti-mutagenic, anti-proliferative, anti-inflammatory and anti-atherogenic activities (Martí *et al.*, 2016; Chaudhary *et al.*, 2018).

Salicylic acid (SA), a natural plant hormone, act as an important signalling molecule triggering tolerance against abiotic and biotic stresses

(Hernández-Ruiz and Arnao, 2018; Gorni *et al*., 2020; Gorni *et al*., 2021). SA plays a significant role in many physiological and biochemical processes of the plant, being able to act as growth regulators and also as an abiotic elicitor capable of increasing the synthesis of secondary compounds beneficial to human health (Javanmardi and Akbari, 2016).

Ferulic acid (FA) is a phenolic compound synthetized from the metabolism of phenylalanine and tyrosine in plants. It is an important biological and structural component of the plant cell wall and it accumulates in soil and influences plant growth (Li *et al.*, 2013; Paiva *et al.*, 2013). FA is considered as non-enzymatic antioxidant and acts under stress to eliminate free radicals produced in plants (Andreasen *et al.*, 2001; Engwa, 2018). Studies on the exogenous application of FA in plants have demonstrated that this compound acts as an important regulator of several physiological processes related to plant growth and mitigating stress, such as, stomatal closure, cell division, membrane permeability, photosynthesis, respiration and many other metabolic processes (Santos *et al.*, 2008; Li *et al.*, 2013; Singh and Deen, 2014; Hussain *et al.*, 2017; Cheng *et al.*, 2018).

However, exogenous SA (Kazemi, 2014) and FA (Singh and Deen, 2014) applications were effective in inducing the growth and formation of secondary metabolites in tomato plants. Therefore, it is suggested that the application of SA and FA may result in combined effects of growth promotion and induction of biosynthetic pathways of secondary metabolism. Previous studies have revealed the hormonal and eliciting action of SA and FA (1.0 mmol L^{-1}) in tomato plants (Hussain *et al*., 2017; Kumar *et al*., 2017). In this context, the application of SA and FA can improve the productive performance and the biosynthesis of secondary compounds in tomato, making possible an increase in the commercial value of this crop, reaching greater market competitiveness. In this study we evaluated the effect of leaf spray of SA and FA either alone and in combination on the growth, yield and secondary compounds in tomato cultivar Santa Clara.

2. Materials and Methods

The experiment was conducted under greenhouse (without temperature and humidity control) covered with a 50% solar radiation shade located in Gammon Colleges, Paraguaçu Paulista (22°41'76" S, 50°58'33" W, 517 a.s.l.), Sao Paulo, Brazil.

Trademark tomato cultivar Santa Clara seeds were placed in a germination tray and after the training period (30 days) they were planted in 18 L pots containing soil. Soil samples were collected and submitted to chemical analysis according to Van Raij *et al.* (2001) (Table 1) and was corrected by applying limestone to increase the saturation of bases to 80% (40.0 g $pots^{-1}$), potassium chloride (3.0 g $pots^{-1}$) and simple super phosphate (20.0 g $pots^{-1}$). For fertilization with micronutrients it was added Yoorin Master 1 Si® (granulated) (Si: 10%, B: 0.1%, Mn: 0.3%, Cu: 0.05% and Zn: 0.55%) (1.5 g $pots^{-1}$), according to the recommendations of Bulletin 100 (IAC) for tomato species. The pots were irrigated by sprinklers per day at 8 a.m. and 5 p.m. in order to keep the soil moisture and ensure the availability of water throughout the experimental period.

The application of salicylic acid (SA: 138.121 g mol^{-1}) and ferulic acid (FA: 194.18 g mol^{-1}) was made for 3 consecutive days after having reached 20 days from transplant. Foliar applications of SA and FA at a dose of 1.0 mmol L^{-1} alone and in combination, and water-treated plants were used as control. SA and FA treatments were carried out by spraying the shoots of the plants with water-based solutions supplemented with Agral® (50 µL L^{-1} of solution) until the drop point (10 mL per plant), as follows: T1 - plants sprayed only with water (Control); T2 - applications of 1.0 mmol L^{-1} SA (SA); T3 - applications of 1.0 mmol L^{-1} FA (FA); and T4 - applications of 1.0 mmol L^{-1} SA + 1.0 mmol L^{-1} FA (SA + FA) by spraying the plants for three consecutive days.

Plants were harvested 90 days after transplanting the seedlings by collecting leaves, roots and fruits.

Plant growth

The effect of SA and FA on the plants were evalu-

Table 1 - Chemical analysis of the soil used in the experiment

pH	Organic matter g dm^{-3}	H + Al mmolc dm^{-3}	Ca mmolc dm^{-3}	Mg mmolc dm^{-3}	K mmolc dm^{-3}	P mg dm^{-3}
4.4	6.0	20.0	7.0	2.0	0.8	6.0

ated based on the fallowing: leaf area (cm^2), number of leaves per plant, number of fruits per plant, plant height (cm), dry weight of shoot and roots (g $plant^{-1}$) and mean fruit weight (g $plant^{-1}$). The leaf area was assessed using ImageJ® Software (Powerful Image Analysis) with 5 plants per treatment. The number of leaves and fruit per plant was determined by manual counting, considering fully expanded leaves and fruits at harvest point. The plant height was determined by measuring tape. The shoot and root dry weight were determined after drying in an oven with air circulation at 70°C until constant weight.

Chlorophyll content

Chlorophyll content (µg mL^{-1}) was determined spectrophotometrically following extraction in acetone, according to the method of Lichtenthaler (1987). Fresh leaf tissue (0.6 g) was added in 7 mL of 80% acetone, the tubes were shaken and left to stand for 30 minutes. The readings were performed in a spectrophotometer at λ 663 and λ 645 nm.

Total soluble sugar content

Total soluble sugar content in fruit was determined according to the method described by Dubois *et al*. (1956) using glucose as the standard. Fresh tissue (0.1 g) was added in 10 mL of 80% ethanol, and macerated in a mortar and left to rest for 30 min. The analyzes followed with the addition of phenol (5%) and sulfuric acid (98 N). The readings were performed in a spectrophotometer at λ 490 nm.

Preparation of fruit tomato extract

Determinations of total polyphenolic compounds and total flavonoid were obtained from the tomato juice. The fruits were ground in a low speed food processor (3000 rpm) for two minutes and passed in 2 mm sieves.

Total polyphenols contents

Total polyphenols contents were determined spectrophotometrically (λ 765 nm) by Folin-Ciocalteu method with modification described by Stagos *et al.* (2012). The total polyphenols contents were reported based on micrograms of gallic acid equivalents per milliliters (µg GAE mL^{-1}). Gallic acid solutions were prepared with concentrations of 25 to 500 µg mL^{-1} in absolute ethyl alcohol. The assays were performed in triplicate.

Total flavonoid content

Total flavonoid content was determined spectrophotometrically (λ 510 nm) by the method of Yao *et al.* (2013). The total flavonoids contents were reported based on micrograms of rutin equivalents per milliliters (µg RE mL^{-1}). Rutin solutions were prepared with concentrations of 25 to 500 µg mL^{-1} in absolute ethyl alcohol. The assays were performed in triplicate.

Carotenoid content

Carotenoid content were extracted from fresh tomato samples after homogenisation of whole fruits. The homogenised sample was mixtured with chloroform/acetone/ethanol (2:1:1, v/v/v) (Sadler *et al.*, 1990). For the determination of lycopene and β-carotene, the absorbance was read at λ 470 and λ 450 nm. Carotenoid content were determined according to the equation described by Craft and Soares (1992), using the molar extinction coefficient of 3450 for lycopene, and 2592 for β-carotene and expressed in micrograms per grams (µg g^{-1}). The assays were performed in triplicate.

Statistical analysis

The experiment was arranged in a completely randomized design with four treatments and five replications per treatment. The Shapiro-Wilk test was used to ensure the normality assumption and the homogeneity of variances. The data were submitted to analysis of variance ($p \leq 0.05$) and Tukey test ($p \leq 0.01$) using the SISVAR software. The results were presented by means of the treatments and standard error.

3. Results

The results indicated that SA and FA both caused a significant increase of growth in tomato (Table 2). The exogenous application of 1.0 mmol L^{-1} SA resulted in increasing in the accumulation of root dry weight by 61% and total dry weight by 41.3% compared to control plants. The application of 1.0 mmol L^{-1} FA resulted in increasing in shoot dry mass by 52.3% and total dry mass by 52% compared to control plants. However, the interaction between SA + FA resulted in increasing in shoot dry mass by 51.5%, root dry mass by 146.37% and total dry mass by 66.6%, when compared to control plants. Fruit weight increased by 16.5% in plant treated with 1.0 mmol L^{-1} FA.

The exogenous application of SA + FA resulted in increasing the leaf area by 47% and number of leaves by 204.3% compared to control plants (Table 3). Application of SA and FA showed increasing of 101.8

Table 2 - Effect of salicylic acid and ferulic acid on the shoot, root, total dry weight and fruit weight in tomato

Treatments	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Total dry weight (g plant^{-1})	Fruit weight (g plant^{-1})
Control	24.01±1.11 c	4.55±0.83 c	28.57±1.60 c	524.46±2.07 b
SA	33.06±1.59 abc	7.32±0.36 b	40.38±1.60 b	554.29±7.44 b
FA	36.57±2.35 a**	6.84±0.42 bc	43.41±1.86 ab	610.78±11.94 a**
SA + FA	36.38±3.99 a	14.02±1.72 a**	47.59±4.57 a**	525.40±20.23 b
CV (%)	15.78	15.08	12.04	5.39

Different letters indicate significant differences by Tukey's test (p≤0.01).

Table 3 - Effect of salicylic acid and ferulic acid on leaf area, number of leaves per plant, number of fruits per plant and plant height in tomato

Treatments	Leaf area (cm²)	Number of leaves	Number of fruits	Plant height (cm)
Control	7955.4±466.9 b	65.2±3.69 c	20.8±0.86 b	77.4±2.67 ns
SA	8739.6±1054.1 ab	131.6±16.02 b	35.4±3.81 a **	79.6±2.75
FA	10209.8±850.6 ab	174.4±10.49 ab	35.0±3.20 a	88.2±7.20
SA + FA	11691.6±577.1 a *	198.4±10.09 a **	28.6±0.40 ab	77.0±1.04
CV (%)	16.11	16.71	19.89	10.75

Different letters indicate significant differences by Tukey's test (p≤0.01).

and 167.5% for the number of leaves, 70.2 and 68.3% for the number of fruits, when compared with control plants (Table 3). The plant height treated with SA and FA was not changed in relation to control plants.

Chlorophyll *a* content (Table 4) observed in tomato plants treated with SA, FA and the interaction with SA + FA were significantly higher by 26, 12 and 27.6%, respectively compared to control. Chlorophyll *b* and total chlorophyll decreased with the application the elicitors (Table 4). The total soluble sugar content in fruits, increased up to 36% in 1.0 mmol L^{-1} SA and 163% in 1.0 mmol L^{-1} FA treated plants when compared to the control.

The exogenous application of SA, FA and the interaction with SA + FA resulted in increasing in the accumulation of total polyphenols of 20.4, 110.3 and 12%, in relation to control plants (Table 5). Total flavonoid content in tomato were 81, 107 and 37% higher in SA, FA and the combined with SA + FA treatments when compared to control plants, respectively. In relation to carotenoid content plants treated with SA also presented increases of 157.3% (lycopene), and 157.7% (β-carotene). The combination of SA+FA showed increases of 65.25% (lycopene), and 65.3% (β-carotene), respectively, in comparison to the control (Table 5).

Table 4 - Effect of salicylic acid and ferulic acid on the chlorophyll and fruit total soluble sugar contents in tomato

Treatments	Chlorophyll *a* (μg mL^{-1})	Chlorophyll *b* (μg mL^{-1})	Total chlorophyll (μg mL^{-1})	Fruit total soluble sugar (mg g^{-1})
Control	9.66±0.23 c	21.02±0.13 a **	30.68±0.14 a **	22.63±0.96 c
SA	12.13±0.07 a	15.69±0.02 c	27.88±0.04 c	30.77±1.38 b
FA	10.82±0.04 b	13.03±0.08 d	23.90±0.05 d	59.47±1.92 a **
SA + FA	12.33±0.07 a **	16.05±0.02 b	28.45±0.04 b	26.89±0.21 bc
CV (%)	1.73	0.73	0.59	7.30

Different letters indicate significant differences by Tukey's test (p≤0.01).

Table 5 - Pair-wise genetic distance estimates based on observed phenotypes of 21 *Amaranthus* accessions

Treatments	Polyphenols (μg GAE mL^{-1})	Flavonoid (μg RE mL^{-1})	Lycopene (μg g^{-1})	β-carotene (μg g^{-1})
Control	245.58±7.21 c	272.33±4.81 d	7.54±0.16 c	10.03±0.2 c
SA	295.58±12.02 b	493.16±2.40 b	19.4±0.16 a**	25.85±0.2 a**
FA	516.42±0.00 a**	564.00±4.81 a**	6.96±0.16 c	9.25±0.2 c
SA + FA	274.75±4.81 b	372.33±4.81 c	12.46±0.00 b	16.58±0.0 b
CV (%)	2.60	1.85	2.39	2.17

Different letters indicate significant differences by Tukey's test (p≤0.01).

4. Discussion and Conclusions

Tomato plants treated with SA and FA presented higher total biomass (Table 2). The effect of elicitors plays a key role in growth regulation, plant development and these findings can be associated with the changes in hormone functions or the improvement of photosynthesis and carbohydrate accumulation in plants (Hussain *et al.*, 2017; Gorni *et al.*, 2020). The foliar application of SA and FA trigger a greater cellular activity that result in a larger number of leaves and leaf area, thus presenting a larger photosynthetic surface, improving the physiological processes (Gorni and Pahceco, 2016; Hussain *et al.*, 2017). Regarding the increase of leaf area, number of leaves, number of fruits and fruit weight, our results showed that the application of SA and FA can alter the growing pattern, in tomato plants (Table 3). The application of SA and FA increased the growth of leaves and roots, without changing the plant height (Khan *et al.*, 2003; de Carvalho *et al.*, 2020). Biomass gains as an effect of SA application were also observed in *Achillea millefolium* (Gorni and Pacheco, 2016), *Foeniculum vulgare* (Gorni *et al.*, 2017) and *Mentha spicata* (Kundu *et al.*, 2018). Increase in biomass, were also observed after FA application in plants of *Arabidopsis thaliana* (Reigosa and Pazos-Malvido, 2007), *Pisum sativum* (Orcaray *et al.*, 2011) and *Cucumis sativus* (Li *et al.*, 2013).

Pigments are the essential components for growth and development and they can determine the health status of plants (Hussain *et al.*, 2017; Gorni *et al.,* 2020). Based on our results, the application the elicitors SA and FA alone or in combination suggest an increase in chlorophyll *a* (Table 4). On the other hand, the application of SA and FA did not affect the chlorophyll *b* and total chlorophyll content, which directly reflected in the plant height values (Table 3). Anway, other studies conducted on different species reported increases in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents consequences of SA treatment (Kazemi, 2014; Chakraborty *et al*., 2016) and in plants sprayed with FA (Zhu and Wakisaka, 2018). Positive effects of SA and FA on the physiological processes of tomato suggest that these elicitors may be associated with the regulation of several essential primary metabolic processes, including synthesis of chlorophylls (Zhu and Wakisaka, 2018; Gorni *et al*., 2020).

The positive effects of the application of SA and FA on the physiological processes of tomato plants were also reflected in higher soluble sugar content (Table 4). Sugar is one of the ingredients in tomato (Hafeznia *et al.*, 2014), and it is an important organic solute with low molecular weight in higher plants. The sugars which are accumulated under stress conditions or as consequence of treatments with SA or FA, keeping osmotic regulation and turgor inside the plant (Orcaray *et al.*, 2011; Gorni *et al.,* 2020). Results show that application of SA also resulted in increasing the total soluble sugar content in tomato plants (Kazemi, 2014), and chamomile (Zarinkamar *et al.*, 2013); similarly to application of FA in pea (Orcaray *et al.*, 2011) and soybean (Ferrarese *et al.*, 2001).

Polyphenols and flavonoids provide many physiological functions for plant survival and are of fundamental importance for plants adaptation (Verma and Shukla, 2015; Mehta *et al.*, 2018). Our results suggest that the application of SA and FA may modulate and alter the concentration of polyphenolic compounds (Table 5) in tomato plants. Studies report that the application of SA and FA stimulates the phenylpropanoid pathway, increasing the accumulation of polyphenols and flavonoids in plants (Salvador *et al.*, 2013; Gorni *et al.*, 2021).

Tomato plants have a good free radical scavenging capacity due to the high concentration of lyco-

pene and β-carotene. This pigment, it is the most effective antioxidant which gives color to tomato fruit as maturity progresses (Kumar *et al.*, 2017). Polyphenolic compounds and carotenoid act as antioxidants because they remove singlet oxygen and other free radicals in cells (Shahidi and Ambigaipalan, 2015) due to their ability to donate hydrogen from hydroxyl groups positioned along the aromatic ring to prevent oxidation by radicals free from lipids and other biomolecules (Sousa *et al.*, 2007).

Researchers showed an increase in carotenoid content during ripening due to the gradual degradation of chlorophyll (Adalid *et al.*, 2010). Our results showed that the application of SA and FA may modulate and alter the concentration of lycopene and β-carotene (Table 5) and chlorophyll *a* contents (Table 4) in tomato plants. Thus, our study evidenced that foliar application of SA and FA in tomato crop could enhance its antioxidant activity due to the higher concentration of these bioactive compounds. Our results corroborate with those found by Kumar *et al.* (2017), who also reported that foliar application of SA significantly increased lycopene content of tomato fruits. Singh *et al.* (2010) reported that application of L-phenylalanine and FA increased total polyphenols in pea plants.

Therefore, it can be concluded that application of bioregulators results in higher yield and higher concentration of secondary compounds of *Solanum lycopersicum*.

References

ADALID A.M., ROSELLÓ S., NUEZ F., 2010 - *Evaluation and selection of tomato accessions (*Solanum *section* Lycopersicon*) for content of lycopene, β-carotene and ascorbic acid.* - J. Food Compost Anal., 23(6): 613-618.

ANDREASEN M.F., LANDBO A.K., CHRISTENSEN L.P., HANSEN Å., MEYER A.S., 2001 - *Antioxidant effects of phenolic rye (*Secale cereale *L.) extracts, monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low-density lipoproteins*. - J. Agr. Food Chem., 49(8): 4090-4096.

CHAKRABORTY N., GHOSH S., CHANDRA S., SENGUPTA S., ACHARYA K., 2016 - *Abiotic elicitors mediated elicitation of innate immunity in tomato: an ex vivo comparison*. - Physiol. Mol. Biol. Plants, 22(3): 307-320.

CHAUDHARY P., SHARMA A., SINGH B., NAGPAL A.K., 2018 - *Bioactivities of phytochemicals present in tomato*. - J. Food Sci. Tech., 55(8): 2833-2849.

CHENG Z.Y., SUN L., WANG X.J., SUN R., AN Y.Q., AN B.L., ZHU M.X., ZHAO C.F., BAI J.G., 2018 - *Ferulic acid pretreatment alleviates heat stress in blueberry seedlings by inducing antioxidant enzymes, proline, and soluble sugars*. - Biol. Plant., 62(3): 534-542.

CRAFT N.E., SOARES Jr. J.H., 1992 - *Relative solubility, stability and absorptivity of lutein and beta-carotene in organic solvents*. - J. Agr. Food Chem., 40(3): 431-434.

DE CARVALHO J.S.B., DA SILVA J.P.R., BATISTA R.D.C.M., 2020 - *Use of salicylic acid as an attenuator to the effects of water deficit on basil plants*. - Diversitas J., 5(3): 1561-1574.

DUBOIS M., GILLES K.A., HAMILTON J.K., REBERS P.T., SMITH F., 1956 - *Colorimetric method for determination of sugars and related substances.* - Anal. Chem., 28(3): 350-356.

ENGWA G.A., 2018 - *Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: Source of antioxidants and role in disease prevention.* - BoD-Books on Demand, 7: 49-74.

FERRARESE M.L.L., SOUZA N.E., RODRIGUES J.D., FERRARESE-FILHO O., 2001 - *Carbohydrate and lipid status in soybean roots influenced by ferulic acid uptake.* - Acta Physiol. Plant., 23: 421-427.

GORNI P.H., BROZULATO M.O., LOURENÇÃO R.S., KONRAD E.C.G., 2017 - *Increased biomass and salicylic acid elicitor activity in fennel (*Foeniculum vulgare *Miller).* - Braz. J. Food Technol., 20: e2016172.

GORNI P.H., PACHECO A.C., 2016 - *Growth promotion and elicitor activity of salicylic acid in* Achillea millefolium *L.* - Afr. J. Biotechnol., 15(16): 657-665.

GORNI P.H., PACHECO A.C., LIMA MORO A., SILVA J.F.A., MORELI R.R., DE MIRANDA G.R., PELEGRINI J.M., SPERA K.D., BRONZEL JR J.L., SILVA R.M.G., 2020 - *Salicylic acid foliar application increases biomass, nutrient assimilation, primary metabolites and essential oil content in* Achillea millefolium *L.* - Sci. Hort., 270:109436.

GORNI P.H., PACHECO A.C., LIMA MORO A., SILVA J.F.A., MORELI R.R., DE MIRANDA G.R., PELEGRINI J.M., ZANIBONI C.B., SPERA K.D., BRONZEL JR J.L., SILVA R.M.G., 2021 - *Elicitation improves the leaf area, enzymatic activities, antioxidant activity and content of secondary metabolites in* Achillea millefolium *L. grown in the field*. - J. Plant Growth, 40(4): 1652-1666.

HAFEZNIA M., MASHAYEKHI K., GHADERIFAR F., MOUSAVIZADEH S.J., 2014 - *Tomato morphological and biochemical characteristics in response to foliar applying of salicylic acid*. - Int. J. Biosci., 5(9): 237-243.

HERNÁNDEZ-RUIZ J., ARNAO M., 2018 - *Relationship of melatonin and salicylic acid in biotic/abiotic plant stress responses.* - Agronomy, 8(4): 33.

HUSSAIN I., SINGH N.B., SINGH A., SINGH H., SINGH S.C., YADAV V., 2017 - *Exogenous application of phytosynthesized nanoceria to alleviate ferulic acid stress in* Solanum lycopersicum. - Sci. Hortic., 214: 158-164.

JAVANMARDI J., AKBARI N., 2016 - *Salicylic acid at differ-*

ent plant growth stages affects secondary metabolites and phisico-chemical parameters of greenhouse tomato. - Adv. Hort. Sci., 30(3): 151-157.

KAZEMI M., 2014 - *Effect of foliar application with salicylic acid and methyl jasmonate on growth, flowering, yield and fruit quality of tomato.* - Bull. Env. Pharmacol. Life Sci., 3(2): 154-158.

KHAN W., PRITHIVIRAJ B., SMITH D.L., 2003 - *Photosynthetic responses of corn and soybean to foliar application of salicylates.*- J. Plant Physiol., 160(5): 485-492.

KUMAR S., YADAV A., YADAV M., YADAV J.P., 2017 - *Effect of climate change on phytochemical diversity, total phenolic content and* in vitro *antioxidant activity of* Aloe vera *(L.) Burm. f*. - BMC Res. Notes, 10(1): 60.

KUNDU M., HALDER S., BHATTACHARJEE A., 2018 - *Salicylic acid induced modulation of growth and metabolism of a medicinal plant* Mentha spicata *L.* - Int. J. Pharm. Sci. Res., 9(12): 5294-5300.

LI D.M., NIE Y.X., ZHANG J., YIN J.S., LI Q., WANG X.J., BAI J.G., 2013 - *Ferulic acid pretreatment enhances dehydration-stress tolerance of cucumber seedlings.* - Biol. Plant., 57(4): 711-717.

LICHTENTHALER H.K., 1987 - *Chlorophyll and carotenoids: pigments of photosynthetic biomembranes,* pp. 350-382. - In PACKER L., and R. DOUCE (eds.) *Methods enzymology*. Academic Press, Sandiego, CA, USA, pp. 762.

MARTI R., ROSELLO S., CEBOLLA-CORNEJO J., 2016 - *Tomato as a source of carotenoids and polyphenols targeted to cancer prevention*. - Cancers, 8(6): 58.

MARTÍNEZ-HERNANDEZ G.B., BOLUDA-AGUILAR M., TABOADA-RODRIGUEZ A., SOTO-JOVER S., MARIN-INIESTA F., LOPEZ-GOMEZ A., 2016 - *Processing, packaging, and storage of tomato products: influence on the lycopene content*. - Food Eng. Rev., 8(1): 52-75.

MEHTA N., PATANI P., SINGHVI I., 2018 - *A review on tomato lycopene*. - Int. J. Pharm. Sci. Res., 9(3): 916-923.

ORCARAY L., IGAL M., ZABALZA A., ROYUELA M., 2011 - *Role of exogenously supplied ferulic and p-coumaric acids in mimicking the mode of action of acetolactate synthase inhibiting herbicides*. - J. Agr. Food Chem., 59(18): 10162-10168.

PAIVA L.B.D., GOLDBECK R., SANTOS W.D.D., SQUINA F.M., 2013 - *Ferulic acid and derivatives: molecules with potential application in the pharmaceutical field.* - Braz. J. Pharm. Sci., 49(3): 395-411.

REIGOSA M.J., PAZOS-MALVIDO E., 2007 - *Phytotoxic Effects of 21 plant secondary metabolites on* Arabidopsis thaliana *germination and root growth*. - J. Chem. Ecol., 33: 1456-1466.

SADLER G., DAVIS J., DEZMAN D., 1990 - *Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates*. - J. Food Sci., 55(5): 1460-1461.

SALVADOR V.H., LIMA R.B., DOS SANTOS W.D., SOARES A.R., BÖHM P.A.F., MARCHIOSI R., FERRARESE M.L.L., FERRARESE-FILHO O., 2013 - *Cinnamic acid increases lignin production and inhibits soybean root growth*. - PLoS One, 8(7), e69105.

SANTOS W.D., FERRARESE M.L.L., NAKAMURA C.V., MOURÃO K.S.M., MANGOLIN C.A., FERRARESE-FILHO O., 2008 - *Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action*. - J. Chem. Ecology, 34(9): 1230-1241.

SHAHIDI F., AMBIGAIPALAN P., 2015 - *Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review*. - J. Funct. Foods, 18: 820-897.

SINGH D.P., BAHADUR A., SARMA B.K., MAURYA S., SINGH H.B., SINGH U.P., 2010 - *Exogenous application of L-phenylalanine and ferulic acid enhance phenylalanine ammonia lyase activity and accumulation of phenolic acids in pea (*Pisum sativum*) to offer protection against* Erysiphe pisi. – Arch. Phytopathol. Plant Protect., 43(15): 1454-1462.

SINGH N.B., DEEN S., 2014 - *Allelopathic potential of ferulic acid on tomato*. - Tunis. J. Plant Prot., 9(1): 1-9.

SOUSA C.M.M., SILVA H.R., VIEIRA-JUNIOR G.M., AYRES M.C.C., COSTA C.D., ARAÚJO D.S., CAVALCANTE L.C.D., BARROS E.D.S., ARAÚJO P.B.M., BRANDÃO M.S., CHAVES M.H., 2007 - *Total phenolics and antioxidant activity of five medicinal plants*. - Quím. Nova, 30(2): 351-355.

STAGOS D., PORTESIS N., SPANOU C., MOSSIALOS D., ALIGIANNIS N., CHAITA E., PANAGOULIS C., RERI E., SKALTSOUNIS L., TSATSAKI A.M., KOURETAS D., 2012 - *Correlation of total polyphenolic content with antioxidant and antibacterial activity of 24 extracts from Greek domestic Lamiaceae species.* - Food Chem. Toxicol., 50(11): 4115-4124.

VAN RAIJ B., DE ANDRADE J.C., CANTARELLA H., QUAGGIO J.A., 2001 - *Chemical analysis for evaluation of fertility of tropical soils.* - IAC, Instituto Agronômico de Campinas, Campinas, Brazil.

VERMA N., SHUKLA S., 2015 - *Impact of various factors responsible for fluctuation in plant secondary metabolites.* - J. Appl. Res. Med. Aromat. Plants, 2(4): 105-113.

YAO X., ZHU L., CHEN Y., TIAN J., WANG Y., 2013 - In vivo *and* in vitro *antioxidant activity and α-glucosidase, α-amylase inhibitory effects of flavonoids from* Cichorium glandulosum *seeds.* - Food Chem., 139(1-4): 59-66.

ZARINKAMAR F., SADERI Z., SOLEIMANPOUR S., 2013 - *Excluder strategies in response to Pb toxicity in* Matricaria chamomilla. - Environ. Ecol. Res., 1(1): 1-11.

ZHU J., WAKISAKA M., 2018 - *Growth promotion of* Euglena gracilis *by ferulic acid from rice bran*. - AMB Express, 8(1): 16.

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Reflective benches for improving lighing in residential basil cultivation

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Key words: Cultivation technology, light supplementation, ornamental species, urban cultivation.

Abstract: Light-reflecting covers can help plant development in shaded environments, improving growing conditions in small residential spaces, encouraging the practice of small cultivations of food and ornamental species. Thus, the objective of this work was to evaluate the effect of reflective bench covers on the growth and development of different cultivars of basil, in pot and shaded environment. The treatments consisted of three reflective materials (aluminum thermal reflective mesh, shiny red and white coating on laminates), arranged on the cultivation benches, and two basil cultivars (green and purple). After 22 days under these environments, the number of leaves and nodes, stem diameter, plant height, relative chlorophyll content, fresh and dry masses of the aerial and roots parts were determined. The highest value of reflective photosynthetically active radiation was shown when using white laminate, followed by the red laminate and thermal reflective mesh. The green basil varieties showed higher values for number of leaves, stem diameter, plant height, fresh shoot weight and number of nodes, compared to the purple variety; however, in relation to the relative chlorophyll content, the purple variety was superior. The bright white and red laminate covers positively influence environmental conditions, increasing the reflectance of photosynthetically active radiation, and the development of basil plants in conditions of greater shading.

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1. Introduction

In view of the urban valorization, the well-lit spaces that could be reserved for the cultivation of ornamental plants, fruits, vegetables, or medicinal plants, have been extinguished in the residences of modern society. With the reduction of the sizes of urban lots, the practice of cultivation continues in inadequate spaces, with little or no direct sunlight, limited in containers, arranged in corridors, balconies, garages and even indoors.

In the last decades the consumer has been looking for novelties in the area of ornamental plants (Noordegraaf, 2000) and has been attracted by the use of species with unusual aesthetic potential for cultivation in pots. However, the lack of information for the cultivation of some species in residential spaces, causes the inadequate development of plants to occur and culminate in loss of stimulus on the part of the owners.

Basil (*Ocimum basilicum* L.) belong to the Lamiacea family and it's economic value have being increased due to its multiple uses as aromatic and seasoning species, on pharmaceutical industry, and essential oil production, (Pereira and Moreira, 2011). This species is a vigorous shrub, with potential to also be used as an ornamental, due to its characteristics as the shape of the plant, leaves, flowers, and inflorescences, added to the pleasant aroma and the possibility of direct consumption (França *et al*., 2017).

Studies with basil have been developed to identify agronomic aspects, chemical compounds for the pharmaceutical and cosmetics industry, among others (Pereira and Moreira, 2011). However, research that seeks to assess the potential of culture for the home environment with some light restriction is incipient.

Light is the main element for photosynthesis to occur, enabling the conversion of light energy into organic energy. Therefore, plant growth and development are affected in a complex way by solar radiation (Taiz *et al*., 2017). Researches have been carried out to evaluate the responses of plants to light intensity, using parameters such as height, diameter of the neck, dry matter of the aerial part and roots. In a study by Chang *et al*. (2008) with four levels of solar radiation on basil, there was a reduction in height, weight, leaf area and an intense drop in photosynthesis under 75% of shading.

In the presence of light, the responses of the plants are mediated by changes depending on the intensity, quality, direction, and duration of the solar incidence and are controlled by specialized photoreceptors (Kami *et al*., 2010). Photoreceptor proteins have a small cofactor or chromophore molecule, which capture and act at specific wavelengths of light over a specific spectrum (Burgie *et al*., 2014).

The development of some plants common to domestic cultivation, such as basil, is effectively affected by both the spectrum and the intensity of light that falls on the plant, which can enhance the biometric development and the chemical composition of plant organs (Stagnari *et al.*, 2018). Thus, the implementation of techniques to obtain better conditions for the development of plants is necessary, since shading interferes with the use of solar radiation causing losses in the development of some species (Conforto *et al*., 2011).

Aiming to obtain concise data and to stimulate the practice of small domestic crops of food and ornamental species cultivation, in environments with low solar incidence, the objective of this work was to evaluate the effect of reflective materials on the growth and development of two basil cultivars potted in a shaded environment.

2. Materials and Methods

Location and characterization of the experimental area

The work was carried out in an urban residential area in the space of a corridor formed between the wall and the house, in the municipality of Cassilândia - MS (19° 10' 81" S; 51° 73' 44" W and average altitude of 540 m), from October to December 2019. According to the Köppen climate classification, the region's climate is humid tropical (Aw) with an average annual temperature of 29°C. During the conduction of the experiment, the climatic data were collected in a meteorological station present in the municipality (Fig. 1).

The space presented between the wall (2.20 m high) and the house wall (6.50 m high) in the dimensions (1.45 m wide and 6.20 m long) forms an open corridor, which receives at least 3 hours of sunlight during the morning (Fig. 2). This environment does not include a source of artificial lighting, the color of the walls of the house being white and the ceramic brick wall in natural color.

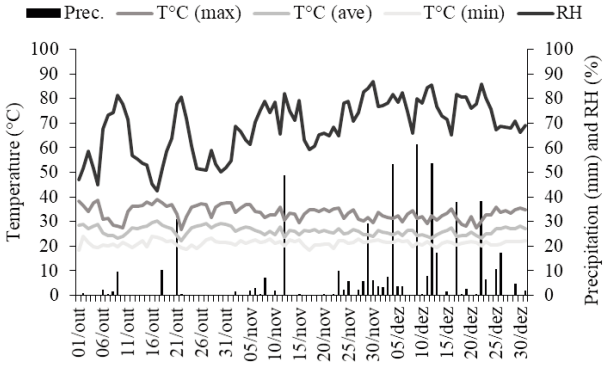

Fig. 1 - Summary of climatic conditions during the experiment.

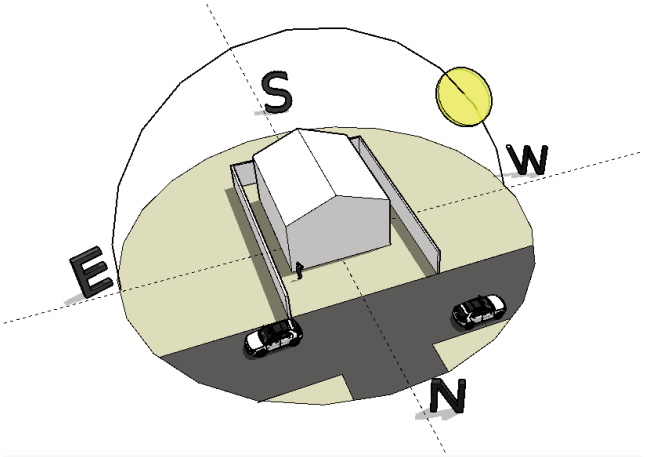

Fig. 2 - Location of the residential corridor used for the domestic cultivation of basil (*Ocimum basilicum* L.) plants in a pot according to the solar orientation.

Experimental design

A completely randomized design was used to evaluate the reflective materials, with six treatments and eight repetitions, each pot being one repetition. The treatments were composed of three reflective materials arranged on the cultivation bench (thermal reflective mesh, bright red laminate sheet; bright white laminate sheet) and two varieties of basil *Ocimum basilicum* L. (sweet basil - green leaf and purple basil - purple leaf), both from the manufacturer Feltrin®.

Implementation and conduction of the experiment

The experiment was installed and conducted on benches made with reused wood pallets, in the dimensions of 0.40 m width, 1.20 m length and 0.90 m height, spaced 0.70 m apart from each other and covered with the three different reflective materials. These same materials were fixed on the walls of the house, in a vertical direction, with dimensions 0.50 m x 1.20 m.

The seedlings were produced in expanded polystyrene trays of 200 cells, filled with commercial substrate CarolinaSoil® at the experimental area. Following the recommendations of the manufacturer, the depth for sowing was 0.5 cm, both for green and purple basil. The emergence occurred 5 days after sowing, thus thinning the seedlings leaving only one per cell and 26 days after sowing the transplant was carried out to the black flexible PVC pots with a capacity of 1 L, being manually irrigated two times a day.

After 22 days, the relative chlorophyll content in the leaves, the number of leaves, plant height, stem diameter, the number of nodes, fresh and dry weight of the aerial part and roots were evaluated.

The plant height, in centimeters, was determined from the level of the substrate in the pots to the inflection of the highest leaf. The stem diameter, in millimeters, was measured from the base of the stem using a digital caliper. The relative chlorophyll content was obtained using a portable digital chlorophyll meter SPAD-502 (Minolta Camera Co. Ltd.), determined from the third or fourth mature leaf and completely expanded, starting from the apex of each plant.

To obtain fresh weight, the plants were cut close to the substrate, divided into aerial part and roots and weighted. The determination of the dry weight of the aerial part and root the plants were dried in an oven with forced air circulation at 65°C for 72 hours.

Microclimatic data of the cultivation environment were collected between 9 am and 10 am on different sunny days, with the Apogee model MP-200 measuring the reflected PAR (µmol m^{-2} s^{-1}) of each material (treatment) with the sensors facing the bench at a height of 30 cm and the incident environmental PAR (µmol m^{-2} s^{-1}).

Statistical analysis

The data were submitted to analysis of variance (Test F) and the averages were compared by the Tukey test, at 5% probability. The analyzes were performed using the statistical software Sisvar® version 5.6 for Windows (Ferreira, 2014).

3. Results and Discussion

It was found that the reflected radiation was higher when using white laminate sheet (Fig. 3), which reflected about 12.67% of the total radiation, followed by the thermal reflective mesh (TRM) and red

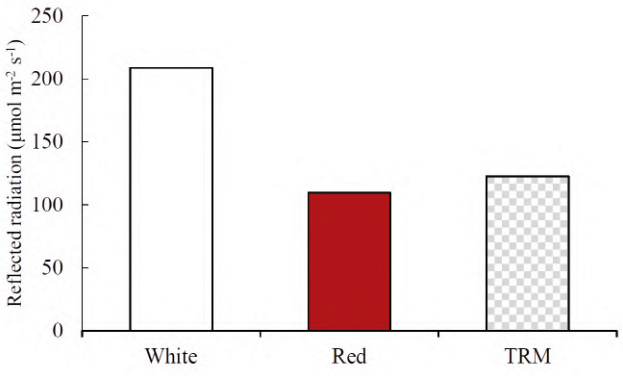

Fig. 3 - Reflected radiation of with different bench covers, from an environmental incident radiation of 1910 µmol m^{-2} s^{-1}.

laminate sheet, with 7.01% and 6,09%, respectively.

The interaction between the two factors studied (cultivars x bench covers) on the growth attributes of basil was not verified ($p>0.05$). However, there was a significant effect, both for the type of bench used ($p>0.05$) and for the varieties ($p>0.05$).

Regardless of the cultivars, basil plants grown on thermal reflective mesh had lower number of leaves, stem diameter, plant height, relative chlorophyll content, fresh and dry aerial weight than plants grown using white and red laminate sheets (Fig. 4). However, the dry root mass did not vary between different materials. Proportionally, these reductions were on average 33.48%, 15.32%, 12.88%, 20.70%, 13.98% and 35.67% respectively, for the number of leaves, stem diameter, plant height, relative chlorophyll content, fresh weight and dry weight of aerial parts, compared to the combined average of white and red laminate sheets (Fig. 4).

The gains in biometric characteristics when using bright white laminate sheet may be related to the quality of light, Antonopoulou *et al*. (2004) state that white light is a rearrangement of colors including blue and red. These wavelengths are those used by plants for the process of photosynthesis, as well as physiological process.

The number of leaves was affected by cover material used, so that plants grown in the environment with bright white and red laminate sheets had a higher number of leaves in relation to the thermal reflective mesh. Therefore, it can be inferred that basil plants are responsive to the quality of light under the conditions evaluated.

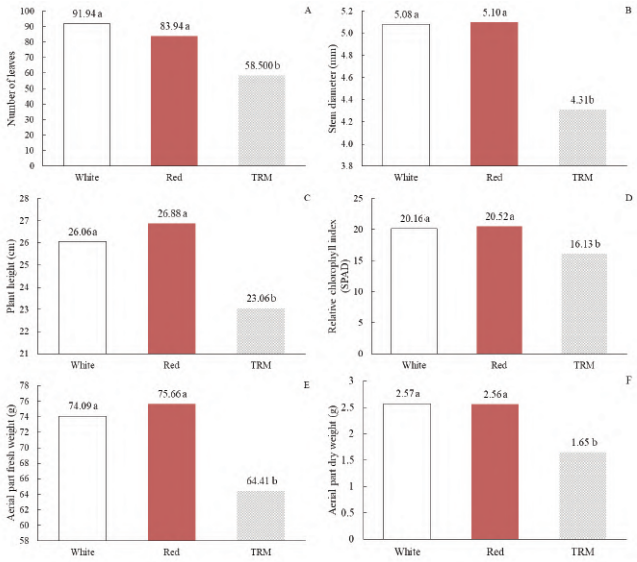

Fig. 4 - Number of leaves (A), stem diameter (B), plant height (C), relative chlorophyll content (D) fresh weight of the aerial part (E) and dry weight of roots (F) of green and purple basil (*Ocimum basilicum* L .), grown in pots in a domestic environment, with different bench covers.

In the cultivation of *Pereskia aculeata*, Vieira (2017) obtained a lower number of leaves under red mesh in relation to the pearl mesh, which is a variation of the white color, showing different results from that found in the present work.

Differences were verified for the stem diameter as a function of the materials used. The thermal reflective mesh provided the lowest value, when compared to the bright white and red laminate sheets. Larger stem diameter is a desirable characteristic in seedlings because it guarantees greater support for the aerial part, when these plants are exposed to adverse wind or intense rain conditions (Souza *et al*., 2014).

The growth pattern of basil plants varied according to the environments to which they were submitted. Plants exposed to colored laminate sheets had greater height when compared to those on thermal reflective mesh but there was no significant difference between white and red laminate sheet.

In a study by Souza *et al*. (2014) evaluating the growth of rosemary plants under colored meshes and in full sun, found a greater height of plants when cultivated in full sun, attributing to this the high luminous intensity and the characteristics of the species. This corroborate with what was observed in the present study, when the high radiation reflected by the white laminate sheet was verified, increasing the total radiation on plant tissues (Fig. 4). Although the red laminate sheet reflected a lower percentage of radiation, it yielded results similar to those of the white laminate sheet, which possibly has a relationship with the quality of the reflected light.

The growth in terms of stem diameter and plant height and higher chlorophyll content in the leaves showed greater gains in the colored laminate sheets, compared to the thermal reflective mesh, indicating that there was no etiolation. According to Taiz *et al.* (2017), etiolated plants have long hypocotyls, a hooked apex, and non-photosynthetic leaves.

Henrique *et al*. (2011), studying the development of coffee seedlings under cover with colored meshes, blue, white, gray, black and red, with shading of 50%, found that the red one was more efficient in promoting growth in plant height, area and leaf dry matter mass and total dry matter mass. In the present study with basil, it was possible to observe that, there was

no difference between the white and red laminate sheets, in the variable plant height.

For the relative chlorophyll the plants placed on the bench covered with thermal reflective mesh had a lower value, which indicates the low capacity of this material to reflect the FRG. The SPAD index (Soil Plant Analysis Development) is used to diagnose the nitrogen status of crops, an element related to plant growth and productivity (Pôrto *et al*., 2011) and related to the photosynthetic condition (Dinh *et al*., 2019).

Costa *et al*. (2012), evaluating the vegetative growth of peppermint seedlings in five environments, full sun and under black, aluminized, blue and red meshes, all with 50% irradiance, observed superior gains in leaf dry weight, stem and shoot biomass under full sun, red and black mesh respectively, when compared to other cultivation environments. Paulus *et al*. (2016) found similar results when cultivating basil under a photoconverting mesh, finding a greater accumulation of fresh and dry mass of the aerial part in full sun, in relation to the red and aluminized mesh. Divergent results were found in the present study, which showed a greater accumulation of fresh weight of the aerial part in plants grown on white and red laminate sheet.

Basil is a heliophyte species and according to Silva (2014) it is considered efficient at high radiation intensities, which promotes better photosynthetic activity. The reflected radiation when using white laminate sheet was higher than that observed for the red one, however, no results were obtained in the accumulation of fresh biomass from the aerial part, which may be also associated with the quality of the light reflected by the materials used.

The aerial part dry weight of the basil plants was affected by the use of the cover materials (Fig. 4F). Plants grown with the use of bright white and red laminate sheets showed a higher dry weight of the aerial part, in relation to the thermal reflective mesh. Melo and Alvarenga (2009), evaluating the effect of radiation in full sun condition and by covering with red, blue and black meshes, in *Catharanthus roseus* L. G. Don vinca plants, found greater increases in dry weight using the red material.

Sunlight is like a shower of photons and plays an essential role in photosynthesis, the process of transforming light energy into chemical energy. Wave spectra have different roles in plants. Blue light (400-500 nm) promotes root growth and intense photosynthesis. The red light that comprises the light range between (600-700 nm, provides increases in the accumulation of dry mass, lengthening of the stem and expansion of the leaf area in addition to improvements in photosynthetic activity. The photoreceptors that promote morphogenic changes in plants are those capable of to absorb blue and red light, with phytochrome being responsible for the absorption of these spectra (Taiz *et al.,* 2017).

Despite the lower proportion of radiation reflected by the red laminate sheet, this did not prevent the plants grown in this environment from accumulating an equal amount of dry weight from the aerial part to that of the plants when grown on white laminate sheet. This demonstrates that plants have the ability to modify their development model in response to the luminous environment (Larcher, 2004).

Plants have a specificity regarding the wavelength received due to the absorption spectrum determined by the photosynthetic pigments present in the chloroplast. The absorption spectrum determines the amount of light energy captured or absorbed by a molecule or substance as a function of the wavelength received. In this sense, the reflective materials arranged on the benches promote better use of radiation by reflecting the luminosity of the environment on the leaf's abaxial face (Lima *et al*., 2018).

Studying the effect of red light emitting diode (LED) lamps on grapes, Poudel *et al*. (2008) found greater optimization of photosynthesis, directly influencing the height of the aerial part, length of internodes and rooting frequency, however, it is noteworthy the need for blue light for chlorophyll synthesis and stomatal activity. Lima *et al*. (2010) evaluating the growth of *Anthurium andraeanum* Apalai under colored meshes with 70% shading found that the black mesh provided better growth conditions in relation to the blue, red and thermal reflective mesh.

Among basil varieties, it was observed that for the variables of leaf number, stem diameter, plant height, fresh shoot weight and number of nodes, the plants of the green variety were 144.20% higher, 34.95%, 35.59%, 4.76% and 20.91%, respectively, in relation to the purple variety. However, in relation to the relative chlorophyll content, the purple variety was 12.0% higher (Fig. 5).

Lin *et al*. (2020) investigating the response of purple and green basil when subjected to different proportions of red, blue and green light, found a greater increase in height, leaf area and stem diameter in green basil plants compared to purple. This shows the influence of the environmental conditions

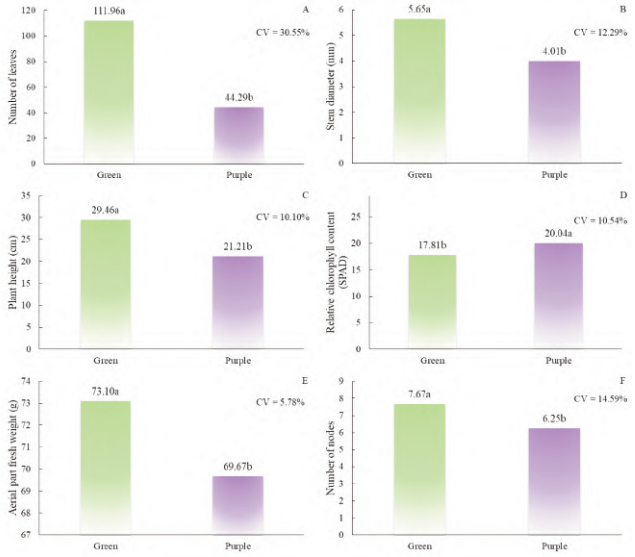

Fig. 5 - Number of leaves (A), stem diameter (B), plant height (C), relative chlorophyll content (D), aerial fresh weight (E) and number of nodes (F) between green and purple basil cultivars (*Ocimum basilicum* L.) grown in pots in a domestic environment, with different bench covers.

imposed on the growth and development of the plants. The results found by the authors are similar to the present study, where there was interference from the environment and the genotype in the growth and development of plants.

The accumulation of dry weight in the aerial part and roots was also higher in plants of the green variety, about 52.51% and 83.64%, respectively, in relation to plants of the purple variety (Fig. 6). The differences between the genotypes of basil used in the present study were expected due to species great genetic diversity, not only morphological, but also in terms of the characteristic compounds of the species,

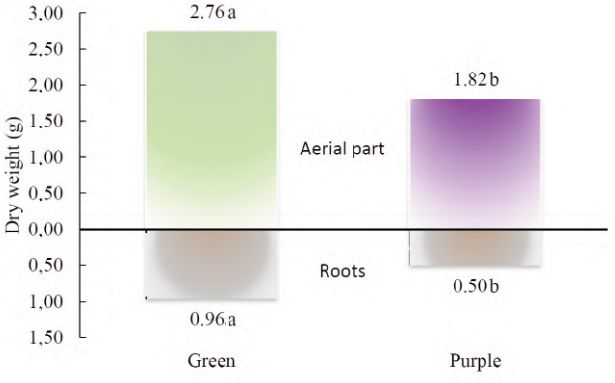

Fig. 6 - Dry weight of the aerial part and roots between green and purple basil cultivars (*Ocimum basilicum* L.), cultivated in pots in a domestic environment, with different bench covers.

such as essential oils (Blank *et al*., 2010; Veloso *et al*., 2014; Castro *et al*., 2016).

The cultivation of plants in homes is a custom passed down from generation to generation. However, due to the growth of urban areas and the consequent reduction of spaces for cultivation, there is a need for innovative techniques that provide better conditions for the development of plants, especially in environments with less incidence of light. In this sense, the use of colored materials on countertops in cultivation environments is a technique that has been studied and has shown positive results in this new model, as demonstrated in the present study.

The use of colored benches in low solar radiation places provides better conditions for the growth and development of basil plants very similar and/or superior to the cultivation carried out in the absence of shading, according to a study by Souza *et al*. (2011). According to the authors, the accumulation of dry weight of plants grown with substrate in full sun was less than the values of the present study.

Based on the results of the present study, and in view of the characteristics of cultivation in domestic spaces, it is inferred preference for the use of white coloring, due to the characteristic of providing greater luminosity to the environment, in addition to the aesthetic enhancement of the vegetables, with contrast provided between the plants and the bench. In addition, concerning the cultivation of basil, the use of a white or red cover favors the development of plants due to the greater reflectance of photosynthetically active radiation. In this way, both colors can be used for the cultivation of this species in shaded environments.

References

ANTONOPOULOU C., DIMASSI K., THERIOS I., CHATZISSAVVIDIS C., 2004 - *The influence of radiation quality on the* in vitro *rooting and nutrient of peach rootstock*. - Biologia Plantarum, 48(4): 549-553.

BLANK A.F., DE SOUZA E.M., DE PAULA J.W., ALVES P.B., 2010 - *Comportamento fenotípico e genotípico de populações de manjericão*. - Horticultura Brasileira, 28: 305-310.

BURGIE E.S., BUSSELL A.N., WALKER J.M., DUBIEL K., VIERSTRA R.D., 2014 - *Crystal structure of the photosensing module from a red/far-red light-absorbing plant phytochrome*. - Proceedings of the National Academy of Sciences, 111(28): 10179-10184.

CASTRO H.G., VELOSO R.A., CARDOSO D.P., CHAGAS

JÚNIOR A.F., CHAGAS L.F.B., 2016 - *Characterization botanical-agronomic and essential oil of basil accessions*. - Revista Verde de Agroecologia e Desenvolvimento Sustentável, 11(4): 1-6.

CHANG X., ALDERSON P.G., WRIGHT C.J., 2008 - *Solar irradiant ce level alters the growth of basil (*Ocimum basilicum *L.) and its content of volatile oils.* - Environ. Exper. Bot., 63: 216-223.

CONFORTO E.C., BITTENCOURT JÚNIOR N.S., SCALOPPI JÚNIOR E.J., MORENO R.M., 2011 - *Comparação entre folhas sombreadas de sete clones adultos de seringueira*. - Revista Ceres, 58(1): 29-34.

COSTA A.G., CHAGAS J.H., PINTO J.E.B.P., BERTOLUCCI S.K.V., 2012 - *Crescimento vegetativo e produção de óleo essencial de hortelã-pimenta cultivada sob malhas*. - Pesquisa Agropecuária Brasileira, 47(4): 534-540.

DINH T.H., TAKARAGAWA H., WATANABE K., NAKABARU M., KAWAMITSU Y., 2019 - *Leaf photosynthesis response to change of soil moisture content in sugarcane*. - Sugar Tech, 21(6): 949-958.

FERREIRA D.F., 2014 - *Sisvar - a Guide for its boots rap procedures in multiple comparisons.* - Ciência e Agrotecnologia, 38(2): 109-112.

FRANÇA M.F.M.S., VILELA M.S., COSTA A.P., NOGUEIRA I., PIRES M.C., SOUZA N.O.S., 2017 -*Germination test and ornamental potential of diferente basil cultivars* (Ocimum spp.). - Ornamental Horticulture, 23(4): 385-391.

HENRIQUE P.C., ALVES J.D., DEUNER S., GOULART P.F.P., LIVRAMENTO D.E., 2011 - *Aspectos fisiológicos do desenvolvimento de mudas de café cultivadas sob telas de diferentes colorações*. - Pesquisa Agropecuária Brasileira, 46(5): 458-465.

KAMI C., LORRAIN S., HORNITSCHEK P., FANKHAUSER C., 2010 - *Light-regulated plant growth and development.* - Current Topics in Developmental Biology, 91: 29-66.

LARCHER W., 2004 - *Ecofisiologia vegetal*. - Vol. I São Carlos, SP, RiMA Artes e Textos, pp. 531.

LIMA J.D., NOMURA E.S., FUZITANI E.J., SILVA S.H.M.G., 2010 - *Variáveis fisiológicas de antúrio cultivado sob diferentes malhas de sombreamento*. - Scientia Agraria, 11(3): 193-200.

LIN K.H., HUANG M.Y., HSU M.H., 2020 - *Morphological and physiological response in green and purple basil plants (*Ocimum basilicum*) under different proportions of red, green, and blue LED lightings*. - Scientia Horticulturae, 275(3).

MELO A.A.M., ALVARENGA A.A., 2009 - *Sombreamento de plantas de* Catharanthus roseus *(L.) G. Don 'Pacifica White' por malhas coloridas: desenvolvimento vegetativo*. - Ciência e Agrotecnologia, 33(2): 514-520.

NOORDEGRAAF C.V., 2000 - *An approach to select new ornamental crops*. - Acta Horticulturae, 541: 75-78.

PAULUS D., VALMORBIDA R., FERREIRA S.B.; ZORZZI I.C., NAVA G.A., 2016 - *Biomassa e composição do óleo essencial de manjericão cultivado sob malhas fotoconversoras e colhido em diferentes épocas.* - Horticultura Brasileira, 34(1): 46-53.

PEREIRA R.C.A., MOREIRA A.L.M., 2011 - *Manjericão: cultivo e utilização.* - Embrapa Agroindústria Tropical, Fortaleza, Brazil, pp.31.

PÔRTO M.L., PUIATTI M., FONTES P.C.R., CECON P.R., ALVES J.C., ARRUDA J.A., 2011 - *Índice SPAD para o diagnóstico do estado de nitrogênio na cultura da abobrinha*. - Horticultura Brasileira, 29(3): 311-315.

POUDEL P.R., KATAOKA I., MOCHIOKA R., 2008 - *Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes*. - Plant Cell, Tissue and Organ Culture, 92(2): 147-153.

SILVA D.F., 2014 - *Utilização de malhas de sombreamento coloridas na produção de mudas e frutos de espécies do gênero* Physalis L. - M.S. Thesis. Lavras Federal University, Brazil, pp. 64.

SOUZA G.S., SILVA J.S., OLIVEIRA U.C., SANTOS NETO R.B., SANTOS A.R., 2014 - *Crescimento vegetativo e produção de óleo essencial de plantas de alecrim cultivadas sob telas coloridas*. - Bioscience Journal, 30(1): 232-239.

SOUZA N.H., CARNEVALI T.O., RAMOS D.D., SCALON S.P.Q., MARCHETTI M.E., VIEIRA M.C., 2011 -*Produção de mudas de manjericão (*Ocimum basilicum *L.) em diferentes substratos e luminosidades.* - Revista Brasileira Plantas Medicinais, 13(3): 276-281.

STAGNARI F., DI MATTIA C., GALIENI A., SANTARELLI V., D'EGIDIO S., PAGNANI G., PISANTE M., 2018 -*Light quantity and quality supplies sharply affect growth, morphological, physiological and quality traits of basil.* - Industrial Crops and Products, 122: 277-289.

TAIZ L., ZEIGER E., MOLLER I.M., MURPHY A., 2017 - *Fisiologia e desenvolvimento vegetal*. Vol. VI. Artmed, Porto Alegre, Brazil, pp. 888.

VELOSO R.A., CASTRO H.G., BARBOSA L.C.A., CARDOSO D.P., CHAGAS JÚNIOR A.F., SCHEIDT G.N., 2014 - *Teor e composição do óleo essencial de quatro acessos e duas cultivares de manjericão (*Ocimum basilicum *L.)*. - Revista Brasileira de Plantas Medicinais, 16: 364-371.

VIEIRA J.S., 2017 -*Propagação vegetativa, crescimento e teor de proteína em ora-pro-nóbis Pereskia aculeata Miller - cultivado sob telas fotosseletivas*. - M.S. Thesis, Federal Institute of Education, Science and Technology of Goias, Brazil, pp. 64.

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Alleviation the effects of salinity stress using titanium dioxide nano and bulk particles in Echinacea seeds and seedlings

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Key words: Abiotic stress, germination percentage, medicinal plant, salt.

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Abstract: This study aimed to investigate the effect of nanoparticles and non-nanoparticles of titanium dioxide on germination indices of Echinacea under salinity stress. Experimental treatments included nano and bulk particles of titanium dioxide at concentrations of 0, 10, 50, 100 and 150 mg/l and salinity stress from NaCl at levels of 0, -3, -6 and -9 bar. The results showed that Echinacea is sensitive to high salinity stress levels (-6 and -9 bar). The use of nano and non-nano titanium dioxide treatment improved some traits under severe salinity stress. The germination percentage did not occur at salinity levels of -6 and -9 bar, but the addition of nano titanium dioxide with a concentration of 150 mg/l and 50 mg/l non-nano increased germination by 50.6%. Application of nano titanium dioxide increased the seedling weight in control by 1.28 mg to 4.26 mg in the treatment of 150 mg/l nanoparticles. The application of nano and bulk titanium dioxide could significantly reduce the negative effect of high salinity stress levels. This can be a valuable and hopeful solution to solve the problem of salinity stress in Echinacea.

1. Introduction

Echinacea (*Echinacea purpurea*) is a perennial herbaceous plant of the chicory family (Asteraceae) and is native to the rocky areas, highlands and Atlantic plains of North America and Canada (Raman *et al*., 2004). The most important medicinal property of this plant, a selected plant of the World Health Organization, is to strengthen the immune system (Sun *et al*., 1999). This plant contains valuable active ingredients such as flavonoid compounds, alkaloids and chicoric acid (Sandra, 2004). The most important substances in Echinacea are essential oils of borneol and alpha-pinene (Faravani *et al*., 2016). In recent years, low rainfall and uncontrolled withdrawal of groundwater resources in the country, followed by an increase in groundwater salinity, has become a significant problem for agriculture, which in addition to reducing fresh water resources, has also increased soil salinity. Therefore, using new technolo-

gies to eliminate and reduce the effects of salinity stress on plants is inevitable. One of the advanced technologies that can be used in this field is the use of nanoparticles. Undoubtedly, by taking advantage of nanotechnology as an emerging advanced technology in the agricultural sector, desirable results can be achieved, including ensuring food security and the development of sustainable and environmentally friendly agriculture in developing countries and regions of the world (Kamali *et al*., 2018).

One of the most important nanoparticles that has been widely used in various sciences is titanium dioxide nanoparticles. Titanium dioxide nanoparticles appear to stimulate plant root and shoot growth by stimulating plant metabolism and increasing cell division. A study by Karami and Sepehri (2018) stated that the application of titanium dioxide nanoparticles improved the growth and photosynthetic performance of barley under salinity stress. This improvement was reported due to increased antioxidant activity in the presence of nanoparticles. Navarro *et al*. (2008) stated that nanoparticles might create new larger pores in the seed coat that facilitate the entry of water and oxygen and increase seed germination. Faraji and Sepehri (2019) showed that the application of titanium dioxide nanoparticles increased germination and morphological traits of wheat seedlings under drought stress. Under moderate and severe stress conditions, the application of titanium dioxide and nitroprusside nanoparticles alone or in combination improved the average germination time of wheat seeds by 56%. The positive effects of titanium dioxide nanoparticles on increasing plant growth, antioxidant enzyme activity, soluble sugars, amino acid and proline content and reduction of H_2O_2 and melonic dihydrogenase in beans under salinity stress have been reported (Abdel Latef *et al*., 2018). Khan (2016) reported a decrease in salinity stress in tomatoes by foliar application of titanium dioxide nanoparticles at a concentration of 20 ppm by improving agronomic traits, leaf chlorophyll content, phenolic and antioxidant capacity, antioxidant enzyme activity and fruit yield. Titanium can act as a stimulant for the plant, activating the immune system against stress. Feizi *et al.* (2020) showed that application of 300 ppm of titanium dioxide nanoparticles improved the mean germination time (MGT) and seed germination rate of lentil by 39% and 62%, respectively. Nasir Khan (2016) reported using 20 mg/lit nano-TiO_2 on tomato plant improved activities of carbonic anhydrase, nitrate reductase, SOD and POX and accumulation of proline and glycine betaine in salinity stress condition.

Gohari *et al*. (2020) showed that the application of titanium dioxide nanoparticles offset the adverse effects of salinity stress on agronomic traits of *Dracocephalum moldavica*. Application of 100 mg/l titanium dioxide nanoparticles under salinity stress of 50 and 100 mM sodium chloride increased the activity of antioxidant enzymes and decreased H_2O_2 concentration. The results of a study on the effect of osmotic and salinity stress on germination and seedling growth indices of *Echinacea purpurea* and *Cynara scoolymus* showed that Echinacea is sensitive to low and medium levels of salinity stress (Amiri *et al*., 2010). Also, with increasing the intensity of osmotic stress, the root length of Echinacea and shoot length of both plants decreased until it reached zero at the levels of -10 and -14 bar for Echinacea and Artichoke, respectively (Amiri *et al*., 2010). In a study on the effect of different osmotic potentials of sodium chloride and calcium chloride salts on the germination characteristics of Echinacea seeds, it was observed that with increasing salt concentration, all germination traits significantly ($p \leq 5\%$) are reduced, so that in the potential of -9 bar, the percentage and rate of germination decreased by 50% and seed vigor by 83% (Ebrahimi Anjeshshi *et al*., 2011). Lyu *et al*. (2017) stated that seeds soaked in titanium dioxide nanoparticles showed a higher germination rate, more root growth and improved seedling growth. Nanoparticles can enter the seed coat and increase the entry of water and nutrients and improve seed growth. But their toxic effects also occur in seeds. Younes *et al*. (2020) reported that the application of 100 ppm of TiO_2 and ZnO nanoparticles on three species of the Solanaceae family significantly improved their germination traits and reduced their average germination time. Kamali *et al.* (2018) showed that at 75 mM salinity, foliar application of titanium dioxide nanoparticles in 15 ppm treatment increased the number of flowers in *Petunia hybrida* plant from 5.6 to 9.3. Also, the highest shoot fresh weight was observed in the treatment of 15, 20 and 40 ppm foliar application of titanium dioxide nanoparticles.

According to research, it seems that the use of nanotechnology can reduce the adverse effects of salinity stress on seeds and plant growth. Therefore, the present study was conducted to investigate the effect of titanium dioxide nano and bulk particles on seed germination and seedling growth of Echinacea in modulating salinity stress conditions.

2. Materials and Methods

In order to investigate the effect of nanoparticles and non-nanoparticles of titanium dioxide on germination indices of Echinacea under experimental salinity stress, an experimental study was performed in the Laboratory of Medicinal Plants of the University of Torbat Heydarieh, Iran. To perform the study, 100 grams of Echinacea seeds were purchased from the Agricultural and Natural Resources Research Center of Isfahan Province. The seeds were carefully threshed and 2700 seeds were isolated for testing. This experiment was conducted as a factorial layout based on a completely randomized design with four replicates.

Each of the experimental steps has 36 integrated treatments, including nine levels of zero (control) titanium dioxide concentration, 10, 50, 100 and 150 mg/l of nanoparticles and 10, 50, 100 and 150 mg/l of bulk particles and four levels of salinity stress were zero (control), -3, -6 and -9 bar in three replications. In these experiments, sterile Petri dishes and filter paper with a diameter of nine cm were used as the culture medium (Top paper culture method or TP).

Exert treatment

The filter papers were wrapped in aluminum foil for each stage of culture and disinfected in an autoclave at 120°C for 20 minutes. Seeds were disinfected using 10-14% sodium hypochlorite for 30 seconds and then washed thoroughly with distilled water three times each time for three minutes until the disinfectant was completely removed from the seed surface. The work surface and all utensils and utensils used were disinfected using 70% ethanol.

In each Petri dish, 25 disinfected seeds were placed at a suitable distance from the bed of filter paper. Then 5 ml of the prepared solutions were added and the lid of the Petri dishes was closed using para film to prevent evaporation of the material and the solution was not added until the end of the test period. Petri dishes were placed in a germinator with a temperature of 25°C and a humidity of 60% at 16/8 hours length in day/night.

Preparing saline and TiO_2 solution

To prepare the final solution of the treatments, each level of titanium dioxide factor must be mixed separately with each of the salinity stress levels in a ratio of 1:1 to obtain a homogeneous solution and then applied to the seeds. For this purpose, titanium dioxide levels, as well as salinity stress, should be prepared in double concentration to achieve the desired concentration after mixing them; Therefore, to prepare 100 ml of each of the concentrations of 20, 100, 200 and 300 mg/l of nanoparticles and non-nano titanium dioxide, the amounts of 5, 25, 50 and 75 ml of stock solution, respectively. It was poured separately and each of them was brought to a volume of 100 ml with the help of distilled water. To prepare different concentrations of salinity, NaCl salt made in Germany (Merck) was used based on Richards method (Richards, 1954). Different salinity levels were prepared with double concentration. Then, 10 ml of titanium dioxide solution was mixed with 10 ml of saline solution at the desired stress level in a Beaker and homogenized.

Nanosized TiO_2 powder was AEROXIDE® TiO_2 P25, supplied by Degussa GmbH Company. Specific surface area of nanosized TiO2 was 50 m^2 g^{-1}, average primary particle size was 21 nm and purity was >99.5%. The size of TiO_2 nanoparticles (Fig. 1) and bulk particles (Fig. 2) were determined. In order to gain accurately dispersed and stable TiO_2 suspensions of each concentration, an ultra-sonication treatment was applied to bulk and nanoparticles TiO_2 powders dispersed in water for 15 minutes.

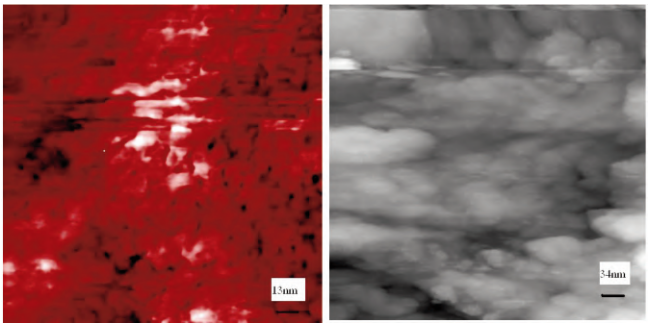

Fig. 1 - Images of nanosized TiO_2 by Scanning Tunneling Microscope (STM).

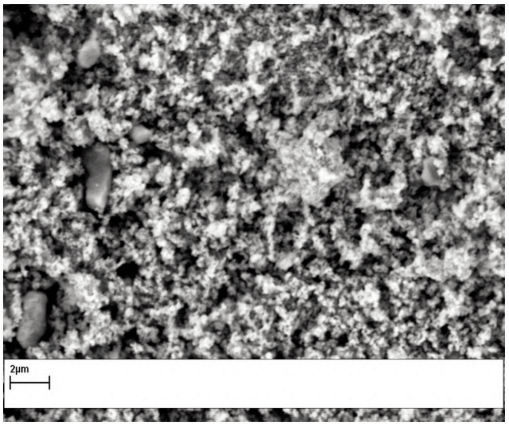

Fig. 2 - Image of bulk TiO_2 particles by Scanning Electron Microscope (SEM).

Measurement methods

Daily counting started from the day after planting at a specific time and continued until the 21st day (ISTA, 2009). Seeds with a root length of more than two millimeters were counted as germinated seeds (ISTA, 2009). At the end of the day, 10 seedlings were randomly selected from each experimental unit and the length of roots, stems and seedlings were measured and recorded using millimeter paper. The stems and roots were separated and each was taken in separate paper bags in an oven at 70°C for 24 hours and then weighed with a digital scale to the accuracy 0.0001 g. To determine the germination rate of Maguire formula (Maguire, 1982), the mean germination time (MGT) (Matthews and Khajeh-Hosseini, 2007) and the mean daily germination (MDG) (Azimi *et al*., 2013) from the following equations used:

$$\text{Germination rate (GR)} = (a/1) + (b-a)/2 + (c-b)/3 + \ldots. [n- (n-1)]/n \quad (1)$$

Where GR indicates the germination rate in terms of germinated seeds per day, a, b, c ... n indicates the number of germinated seeds after N ... 3, 2, 1 day after dewatering.

$$MGT = [\sum(F \cdot X)]/(\sum F) \quad (2)$$

$$MDG = \text{Germination\% / total experiment days} \quad (3)$$

In Equation (2), MGT: mean germination time (days), F: the number of new germinated seeds per day of count X and X days of counting. Equation (4) and (5) were used to calculate the seed vigor index (Vashith and Nagarajan, 2010):

Vigor index I = germination % x seedling length in cm (shoot + seminal root) (4)

Vigor index II = germination % x seedling dry mass in mg (shoot + seminal root) (5)

Data related to excel software were sorted and processed and then statistical analysis of the data was performed by SAS JMP software and comparisons of means were performed by Tukey test at 5% probability level.

3. Results and Discussion

The results of data analysis of variance are reported in Table 1. Application of titanium dioxide treatment had a significant effect on all studied traits except seed vigor index I and shoot length. The effect of salinity stress was significant on all traits except shoot, root and seedling weight. The results also showed that all traits except shoot and seedling weight, seed vigor index I were significantly affected by the interaction of titanium dioxide and salinity stress.

Effect of titanium dioxide on germination indices of Echinacea under salinity stress

As shown in Table 2, the application of titanium dioxide treatment improved the germination percentage and rate and mean daily germination compared to the control. Application of titanium dioxide nanoparticle increased the seedling weight from the control with a value of 1.28 mg to 4.26 mg in the treatment of 150 ppm nanoparticles. The application of titanium dioxide had no effect on the shoot weight of Echinacea. The results of this test are the same as the experimental results performed by Tokalloo *et al.* (2013) on barley and Feizi *et al*. (2013) on sage. All experimental treatments significantly positively affected shoot length, seedling weight, and root weight compared to the control. The lowest root length was related to the control and the concentration of 150 mg/l of non-nanoparticles had the best performance, so that it increased the length of root, stem and seedling about three times compared to the control.

Table 1 - Analysis of variation of nano and bulk titanium dioxide particles on germination and seedling traits of Echinacea

Source of variation	df	Seed vigor II	Seed vigor I	Seedling length	Root length	Shoot length	Seedling weight	Root weight	Shoot weight	Mean daily germination	Mean germination time	Germination rate	Germination
TiO_2	8	117638 **	25137.69 NS	158.72 **	50.52 **	58.85 **	6.99 NS	0.72 **	5.53 NS	1.77 **	21.47 **	1.32 **	781.92 **
Salinity	3	4829442 **	84607.21 **	829.73 **	508.05 **	94.85 **	5.74 NS	0.52 NS	8.74 NS	17.50 **	20.06 **	48.48 **	7542.51 **
TiO2× Salinity	24	434076 **	26985.32 NS	43.58 **	35.76 **	13.92 **	5.63 NS	0.44 **	4.34 NS	1.31 **	11.50 **	0.84 **	587.40 **
Error	72	36544	24260.7	8.85	6.47	2.82	4.35	0.23	4.13	0.27	0.85	0.21	121.93
Total	107												

NS, *, and **: No significant, significant at 5 and 1% probability, respectively.

Table 2 - Effect of titanium dioxide particles on germination traits of Echinacea under salinity stress

TiO_2 concentration mg/l	Vigor index II	Vigor index I	Seedling length (mm)	Root length (mm)	Shoot length (mm)	Seedling weight (mg)	Root weight (mg)	Shoot weight (mg)	Mean daily germination (seed)	Germination rate (seed/day)	Germination (%)
0	601.46 ab	107.05 a	7.16 c	3.19 c	3.97 b	1.28 b	0.16 b	1.12 a	2.03 b	1.75 b	42.66 b
N10	826.53 a	181.13 a	16.79 ab	6.83 ab	9.95 a	2.82 ab	0.84 a	1.98 a	3.19 a	2.44 a	67.00 a
N50	648.57 ab	171.00 a	15.50 b	5.24 bc	10.25 a	2.97 ab	0.89 a	2.08 a	2.79 a	2.59 a	58.33 a
N100	574.85 b	161.40 a	14.00 b	4.09 bc	9.89 a	2.60 ab	0.44 ab	2.15 a	2.93 a	2.75 a	61.66 a
N150	605.16 ab	282.37 a	15.05 b	5.29 bc	9.91 a	4.26 a	0.49 ab	3.76 a	2.96 a	2.56 a	62.00 a
B10	811.69 ab	195.43 a	16.97 ab	6.67 b	10.31 a	2.95 ab	0.80 a	2.15 a	3.19 a	2.75 a	64.33 a
B50	762.01 ab	167.62 a	17.82 ab	5.97 bc	11.93 a	2.57 ab	0.40 ab	2.17 a	3.11 a	2.60 a	65.33 a
B100	769.84 ab	181.81 a	14.17 b	3.89 bc	10.27 a	2.56 ab	0.43 ab	2.13 a	3.33 a	2.81 a	70.00 a
B150	768.81 ab	168.33 a	20.32 a	10.04 a	10.27 a	2.54 ab	0.42 ab	2.12 a	3.20 a	2.82 a	67.33 a

Means, in each column, followed by same letter are not significantly different at the 5% probability level, using Tukey Test. n=nano, b=bulk

The effect of salinity stress on germination indices of Echinacea

The results reported in Table 3 show that Echinacea had the highest germination percentage at the level of zero and salinity stress -3 bar, but with increasing salinity stress, the germination percentage decreased significantly. At the stress level of -9 bar, it decreased by 37.5% compared to the control. The highest germination percentage was related to the control treatment and decreased significantly with increasing salinity stress levels, so that it decreased by 2.5 times compared to the control at -9 bar.

Miri and Mirjalili (2013) stated that one of the ways to priming seed is to use NaCl salt solution with salinity 1 dS m^{-1}, which in saline areas improves seedling growth rate by up to 20%. They stated that at 1 dS m^{-1} salinity the germination rate of Echinacea is 57.7% but with increasing the amount of NaCl to 6 dS m^{-1} salinity the germination rate decreases to about 9.8%.

The highest germination rate was observed in the control treatment and with increasing the intensity of salinity stress, the germination rate decreased significantly. Seeds that are exposed to salinity stress face water shortage, resulting in a decrease in germination rate and percentage under the influence of salinity (Kafi *et al*., 2005). Seed and impaired storage protein synthesis reduce seed germination (Vigot, 2009). In addition, the toxicity of sodium and chlorine ions in salinity stress play an important role in reducing seed germination (Hanslin and Eggen, 2005). In a study on the effect of different osmotic potentials of sodium chloride and calcium chloride salts on the germination characteristics of Echinacea seeds, it was observed that with increasing salt concentration, all germination traits significantly ($p \leq 5\%$) are reduced, so that in the potential of -9 bar, the percentage and rate of germination decreased by 50% and seed vigor by 83% (Ebrahimi Anjeshshi *et al*., 2011). The inhibitory effects of sodium chloride on seed germination may be due to its direct effect on embryo growth. The researchers found that fetal axis elongation was severely inhibited by high levels of sodium chloride in the irrigation solution. On the

Table 3 - Effect of salinity stress on germination traits of Echinacea

Salinity (bar)	Vigor index II	Vigor index I	Seedling length (mm)	Root length (mm)	Shoot length (mm)	Seedling length (mg)	Root length (mg)	Shoot length (mg)	Mean daily germination (seed)	Germination rate (seed/day)	Germination (%)
0	335.32 c	198.97 ab	21.76 a	11.71 a	10.05 b	2.53 a	0.71 a	1.81 a	3.76 a	4.31 a	78.37 a
-3	1288.88 a	245.66 a	18.04 b	6.01 b	12.06 a	3.42 a	0.39 a	3.03 a	3.43 a	2.80 b	72.14 a
-6	741.65 b	159.21 ab	11.41 c	2.68 c	8.73 c	2.49 a	0.49 a	1.99 a	2.70 b	1.97 c	56.74 b
-9	464.78 c	114.43 b	10.02 c	2.36 c	7.72 c	2.47 a	0.57 a	1.90 a	1.95 c	1.17 d	41.03 c

Means, in each column, followed by same letter are not significantly different at the 5 % probability level- using Tukey Test. n=nano, b=bulk.

other hand, sodium chloride, due to inhibition of water uptake by seeds, slows down vital activities in the seed and increases rooting time (Mohammadi *et al*., 2011).

The mean daily germination trait had the best performance at zero and -3 bar the salinity stress level, but decreased significantly with increasing the stress level and reached the lowest level at -9 bar. No significant difference was observed between stem levels in shoot weight, root weight and seedling weight. However, numerically, yield in shoot weight and seedling weight at the level of 3-bar stress showed a slight increase compared to the control, which decreased with increasing stress intensity. The highest shoot length was observed at the level of -3 bar salinity stress, which was higher than the control. The root length in control had the highest value and decreased sharply with increasing the stress level, so that the root length at the level of -9 bar drought load was reduced about 10 times compared to the control. Seedling length was highest at zero stress level, but with increased stress level to -9 bar, seedling length was reduced by half. The results of research on sage showed a decrease in root and stem length with increasing osmotic stress (Stephanie *et al*., 2005). The results of studies on sage and ten species of medicinal plants showed the negative effect of salinity stress on plant length (Fallahi *et al*., 2009).

The best seed vigor index I was seen at the stress level of -3 bar, which was not significantly different from the control level and -6 bar. But compared to the -9 bar stress level, it was about 2 times higher. Seed vigor index II had the best performance at -3 bar stress and a significant decrease was seen at -6 bar level. Seed vigor index II in control and -9 bar stress had the lowest value (Table 3).

Interaction of titanium dioxide and salinity stress on germination indices of Echinacea

As can be seen from the results reported in Table 4, the use of titanium dioxide treatment increased the germination percentage at high salinity stress condition. In the absence of nano titanium dioxide at -6 and -9 bar level salinity stress, no germination occurred at all, but with the application of titanium dioxide, the germination percentage increased significantly in the mentioned stress intensities. So that in the control treatment with salinity stress -9 bar germination was zero; however, in the treatments of 50 mg/l non-nanoparticles and 150 mg/l nanoparticles at the same stress level, germination was observed 50.66%.

Contrary to the results of the present experiment, Zheng *et al*. (2005) reported that titanium dioxide nanoparticles absorb more water in spinach seeds, thus accelerating seed germination. Khot *et al*. (2012) pointed out that the main reason for the increase in plant growth rate in response to titanium dioxide nanoparticles is the production of sterile radiation oxygen, which increases seed resistance to stress and improves water and oxygen penetration in accelerating germination.

In terms of germination rate, the application of titanium dioxide increased the germination rate at high levels of salinity stress compared to the control and the treatment of 10 mg/l of non-nanoparticles maintained the germination rate during increasing salinity stress. However, under non-stress conditions, the concentration of 150 mg/l of non-nanoparticles had a higher performance than all experimental treatments.

The mean germination time in Echinacea seeds decreased with increasing salinity stress level. Due to this, a limited number of seeds germinated at high salinity stress levels in the first days of the experiment and during the counting days, due to severe salinity, the germination process stopped; however, at low stress levels, the germination process continued until the last days of counting, which increased the mean germination time at low salinity stress levels.

The application of titanium dioxide caused the mean daily germination during the increasing process of salinity stress to be higher than the control. In general, increasing the salinity stress level from zero to -9 bar reduced the mean daily germination; however, among the experimental treatments, 150 mg/l nanoparticles and 50 mg/l non-nanoparticles were able to better inhibit the decrease in germination mean due to increased salinity stress.

According to the results reported in Table 5, the best treatment for stem weight was 150 mg/l nanoparticles at a stress level of -3 bar. No significant difference was observed between other experimental treatments; However, treatments of 100 mg/l nanoparticles and 150 mg/l of non-nanoparticles had 6 times higher shoot weight at the stress level. Application of titanium dioxide treatment increased root weight compared to control at high stress levels. There was no significant difference in non-nano and control treatments at different levels of salinity stress in seedling weight.

Table 4 - Interaction effect of titanium dioxide particles and salinity on germination traits of Echinacea

TiO_2 (mg/l)	Salinity (bar)	Mean daily germination (seed)	Mean germination time (day)	Germination rate (seed/day)	Germination (%)
0	0	4.06 ab	6.46 c-g	4.31 abc	85.33 ab
	-3	3.87 a-d	9.04 abc	2.57 d-i	81.33 a-d
	-6	0.19 fg	0.42 h	0.11 jk	4.00 fg
	-9	g 0	h 0	k 0	g 0
10n	0	3.55 a-e	7.28 a-g	3.04 c-g	74.66 a-e
	-3	3.49 a-e	7.36 a-g	2.92 c-h	73.33 a-e
	-6	3.36 a-e	8.95 a-d	2.39 f-i	70.66 a-e
	-9	2.34 b-e	9.48 ab	1.41 h-k	49.33 b-e
50n	0	3.87 a-d	5.39 fg	4.57 ab	80 a-d
	-3	2.98 a-e	7.06 b-g	2.54 e-i	62.66 a-e
	-6	2.41 a-e	6.99 b-g	2.26 f-i	50.66 a-e
	-9	1.90 ef	8.90 a-d	1.23 ijk	40.00 ef
100n	0	4.12 a	6.43 c-g	4.92 a	86.66 a
	-3	3.49 a-e	6.42 c-g	3.19 b-f	73.33 a-e
	-6	2.22 de	8.43 a-e	1.63 g-j	46.66 de
	-9	1.90 ef	8.39 a-f	1.26 ijk	40.00 ef
150n	0	3.36 a-e	4.84 g	4.09 a-d	69.33 a-e
	-3	3.11 a-e	7.34 a-g	2.58 d-i	65.33 a-e
	-6	2.98 a-e	7.58 a-g	2.26 f-i	62.66 a-e
	-9	2.41 a-e	10.18 a	1.31 ijk	50.66 a-e
B10	0	3.49 a-e	5.72 efg	4.33 abc	70.66 a-e
	-3	3.55 a-e	6.98 b-g	3.02 c-g	74.66 a-e
	-6	3.17 a-e	8.63 a-e	2.32 f-i	66.66 a-e
	-9	2.15 de	9.08 abc	1.31 ijk	45.33 d-e
B50	0	3.80 a-d	7.13 b-g	4.56 ab	80.00 a-d
	-3	2.85 a-e	8.53 a-e	2.045 f-i	60.00 a-e
	-6	3.36 a-e	8.60 a-e	2.36 f-i	70.66 a-e
	-9	2.41 a-e	9.40 abc	1.41 h-k	50.66 a-e
100b	0	3.42 a-e	5.96 d-g	3.93 a-e	72.00 a-e
	-3	4.00 abc	7.37 a-g	3.36 b-f	84.00 abc
	-6	3.61 a-e	8.20 a-f	2.66 d-i	76.00 a-e
	-9	2.28 cde	10.18 a	1.30 ijk	48.00 cde
150b	0	4.12 a	6.67 b-g	5.03 a	86.66 a
	-3	3.55 a-e	7.37 a-g	2.95 c-g	74.66 a-e
	-6	2.98 a-e	9.10 abc	1.99 f-i	62.66 de
	-9	2.15 de	9.06 abc	1.32 ijk	45.33 de

Means, in each column, followed by same letter are not significantly different at the 5 % probability level, using Tukey Test. n=nano, b=bulk

Feizi *et al.* (2012) stated that titanium dioxide treatment had no significant effect on shoot dry weight, seedling, vigor index I and II of wheat; However, the application of titanium dioxide at all levels caused a significant increase in root dry weight and the highest root dry weight was observed in the treatments of 2 and 500 mg/l non-nanoparticles and 100 mg/l nanoparticles.

There was a significant difference between control treatment and different concentrations of titanium dioxide in shoot length, especially at high salinity stress levels. In general, in all treatments, -3 bar level salinity stress increased stem length.

Studies on seedling length also showed that the application of titanium dioxide had a significant positive effect on non-use at high stress levels and in most experimental treatments at the level of -3 bar the salinity stress compared to the non-stress state, seedling length increased shows. Treatment of 150 mg/l non-nanoparticles in the process of increasing salinity stress from zero to -9 bar was able to maintain the root length to a higher value. The results of Paravar and Omidi (2014) and Motevasel *et al*. (2014) showed that with increasing salinity stress, seedling

Table 5 - Interaction effect of titanium dioxide particles and salinity on seedling traits of Echinacea

TiO_2 (mg/l)	Salinity (bar)	Vigor index II	Vigor index I	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Seedling weight (mg)	Root weight (mg)	Shoot weight (mg)
0	0	1622.13 ab	215.28 ab	19.03 b-g	10.03 b-e	9.00 b-g	2.52 b	0.45 ab	2.07 b
	-3	783.73 d-i	212.92 ab	9.63 ghi	2.73 d-g	6.90 fg	2.61 b	0.20 ab	2.41 ab
	-6	k 0	b 0	i 0	g 0	h 0	b 0	0.00 b	b 0
	-9	k 0	b 0	i 0	g 0	h 0	b 0	0.00 b	b 0
10n	0	149.85 jk	174.94 ab	19.70 b-f	11.70 b	8.00 c-g	2.30 b	0.81 ab	1.49 b
	-3	1663.06 a	188.41 ab	22.70 bcd	9.30 b-f	13.40 abc	2.56 b	ab0.41	2.15 b
	-6	923.46 c-h	166.25 b	13.13 d-h	4.16 b-g	8.96 b-g	2.34 b	ab0.38	1.95 b
	-9	569.73 f-k	194.92 ab	11.64 fgh	2.18 efg	9.45 b-g	3.08 ab	0.75 ab	2.33 b
50n	0	135.36 jk	197.22 ab	16.93 b-h	7.23 b-g	9.70 a-g	2.46 b	0.85 ab	1.61 b
	-3	1414.00 abc	183.61 ab	22.76 bcd	7.80 b-g	14.96 a	2.91 ab	0.38 ab	2.53 ab
	-6	597.13 f-k	157.61 b	11.75 fgh	2.90 d-g	8.85 b-g	3.06 ab	0.88 ab	2.18 b
	-9	447.80 g-k	145.54 b	10.55 fgh	3.03 c-g	7.51 efg	2.45 ab	0.45 ab	2.00 b
100n	0	169.84 ijk	219.93 ab	19.60 b-f	7.03 b-g	12.56 a-e	2.54 b	0.65 ab	1.88 b
	-3	1243.73 a-e	195.29 ab	17.00 b-h	4.23 b-g	12.76 a-e	2.67 b	0.30 ab	2.36 ab
	-6	437.20 g-k	133.38 b	8.10 hi	1.33 fg	6.73 g	2.77 b	0.33 ab	2.44 ab
	-9	448.64 g-k	97.02 b	11.31 fgh	3.78 b-g	7.52 efg	2.42 b	0.50 ab	1.92 b
150n	0	160.93 ijk	173.66 ab	23.16 bc	11.23 bc	11.93 a-g	2.48 b	0.71 ab	1.77 b
	-3	944.00 c-h	681.26 a	14.43 c-h	4.86 b-g	9.56 a-g	9.71 a	0.70 ab	9.01 a
	-6	840.40 c-h	154.45 b	13.36 d-h	2.86 d-g	10.50 a-g	2.44 b	0.25 ab	2.19 b
	-9	475.33 g-k	120.09 b	9.26 hi	2.20 efg	7.66 d-g	2.40 b	0.32 ab	2.08 b
B10	0	139.44 jk	188.77 ab	19.63 b-f	10.86 bcd	8.77 b-g	2.67 b	0.83 ab	1.84 b
	-3	1632.40 a	202.61 ab	21.86 b-e	9.13 b-f	12.73 a-e	2.70 b	0.48 ab	2.22 b
	-6	896.00 c-h	282.26 ab	13.60 c-h	4.60 b-g	9.04 b-g	3.04 ab	0.78ab	2.25 b
	-9	579.04 f-k	108.09 b	12.81 e-h	2.10 efg	10.70 a-g	2.41 b	0.12 b	2.28 b
B50	0	200.85 ijk	213.36 ab	24.83 b	10.80 bcd	14.03 ab	2.66 b	0.66 ab	1.99 b
	-3	1166.80 a-f	155.04 b	19.33 e-g	6.83 b-g	12.83 a-e	2.68 b	0.37 ab	2.31 b
	-6	1044.00 a-g	173.58 ab	14.93 c-h	2.80 d-g	12.13 a-g	2.49 b	0.21 ab	2.27 b
	-9	636.40 e-j	128.49 b	12.20 e-h	3.46 b-g	8.73 b-g	2.46 b	0.35 ab	2.11 b
100b	0	114.96 jk	198.16 ab	15.93 b-h	7.80 b-g	8.13 c-g	2.74 b	0.78 ab	1.96 b
	-3	1451.60 abc	215.37 ab	17.30 b-h	4.20 b-g	13.10 a-d	2.56 b	0.34 ab	2.22 b
	-6	1003.06 b-g	199.90 ab	12.96 e-h	2.13 efg	10.83 a-g	2.61 b	0.30 ab	2.31 b
	-9	509.74 g-k	113.80 b	10.50 fgh	1.43 fg	9.03 b-g	2.34 b	0.29 ab	2.04 b
150b	0	324.68 h-k	209.42 ab	37.06 a	28.70 a	8.36 c-g	2.41 b	0.70 ab	1.71 b
	-3	1300.66 a-d	176.44 ab	17.33 b-h	5.03 b-g	12.30 a-f	2.38 b	0.30 ab	2.07 b
	-6	933.60 c-h	165.46 ab	14.90 c-h	3.33 c-g	11.56 a-g	2.66 b	0.29 ab	2.37 ab
	-9	516.30 g-k	122.01 b	11.98 fgh	3.10 c-g	8.87 b-g	2.71 b	0.38 ab	2.32 b

Means, in each column, followed by same letter are not significantly different at the 5 % probability level- using Tukey Test. n=nano, b=bulk

length, root length and seedling dry weight decreased.

In Seed Vigor Index II, all treatments containing titanium dioxide, salinity stress -3 bar made a significant positive difference compared to the control, but in the conditions without stress, the control had better performance. At high salinity stress levels, the application of titanium dioxide significantly increased the seed vigor index II compared to the time of non-use.

4. Conclusions

Investigation of the main effect of titanium dioxide treatment in testing the effect of nano and non-nano titanium dioxide on germination of Echinacea under salinity stress showed that the use of this treatment in all concentrations, measured indices (except increased seed vigor indices) increased compared to the control treatment. Echinacea tolerated salinity stress up to -3 bar in germination percentage,

mean germination time, shoot length, seed germination index I and II, and sometimes even at -3 bar stress observed better yield than the control. But germination rate, root and seedling length were strongly affected by salinity stress. The interaction of titanium dioxide and salinity stress treatments showed that although in the increasing trend of salinity stress intensity all studied traits were significantly reduced compared to non-stress conditions, but the application of titanium dioxide treatment in nano and non-nano state in -6 and -9 bar salinity improved significant yield of Echinacea seeds and seedlings in all traits compared to the control. Therefore, the positive effects of using titanium dioxide in mitigating the negative effects of salinity stress on the seeds and seedlings of Echinacea can be a useful and promising solution to solve this problem. Further research is needed to determine the physiological and molecular effects of this substance on the metabolism of plant resistance to salinity stress.

References

ABDEL LATEF A.A., SRIVASTAVA A.K., ABD EL-SADEK M.S., KORDROSTAMI M., TRAN L.-S.P., 2018 - *Titanium dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil conditions*. - Land Degrad. Develop., 29: 1065-1073

AMIRI B., REZVANI MOGHADAM P., AHYAEI H., FALLAHI J., AGHWANI SHAJARI M., 2010 - *Effect of osmotic and salinity stresses on germination and seedling growth indices of* Echinacea purpurea *and* Cynara scoolymus. - J. Environ. Stress Agric. Sci., 3(2): 165-176.

AZIMI R., JANKJU BORZELABAD M., FEIZI H., AZIMI A., 2014 - *Interaction of SiO_2 nanoparticles with seed prechilling on germination and early seedling growth of tall wheatgrass (*Agropyron elongatum *L.)*. - Polish J. Chem. Technol., 16(3): 25-29.

EBRAHIMI ANJESHSHI Z., DASHTI M., SHARIATI M., 2011 - *Effect of different osmotic potentials of sodium chloride and calcium chloride salts on germination characteristics of* Echinacea purpurea *seeds*. - Proc. Second Nat. Conf. on Seed Science and Technology, Islamic Azad University, 4-5 November, Mashhad.

FALLAHI J., EBADI M.T., GHORBANI R., 2009 - *The effects of salinity and drought stresses on germination and seedling growth of clary*. - Environ. Stress Agric. Sci., 1(1): 57-67.

FARAJI J., SEPEHRI A., 2019 - *Ameliorative effects of TiO_2 nanoparticles and sodium nitroprusside on seed germination and seedling growth of wheat under PEG-stimulated drought stress*. - J. Seed Sci., 41: 309-317.

FARAVANI M., GHOLAMI B., FEIZI H., 2016 - *Agronomy of* Echinacea. - University of Torbat, Heydarieh Press, pp. 83.

FEIZI H., AGHELI N., SAHABI H., 2020 - *Titanium dioxide nanoparticles alleviate cadmium toxicity in lentil (*Lens culinaris Medic) seeds. - Acta Agric Slovenica, 116: 59-68.

FEIZI H., AMIRMORADI SH., ABDOLLAHI F., JAHEDI POUR S., 2013 - *Comparative effects of nanosized and bulk titanium dioxide concentrations on medicinal plant* Salvia officinalis *L.* - Ann. Rev. Res. Biol., 3(4): 814-824.

FEIZI H., REZVANI MOGHADDAM P., SHAHTAHMASSEBI N., FOTOVAT A., 2012 - *Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth*. - Biol. Trace Elem. Res., 146(1): 101-106.

GOHARI G., MOHAMMADI A., AKBARI A., PANAHIRAD S., DADPOUR M.R., FOTOPOULOS V., KIMURA S., 2020 - *Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of* Dracocephalum moldavica. - Sci. Rep., 10: 912.

HANSLIN H.M., EGGEN T., 2005 - *Salinity tolerance during germination of seashore halophytes and salt-tolerant grass cultivars*. - Seed Sci. Res., 15: 43-50.

ISTA, 2009 - *ISTA rules*. - International Seed Testing Association, Zuric, Switzerland.

KAFI M., NEZAMI A., HOSSEINI H., MASOUMI A., 2005 - *Physiological effects of drought stress induced by polyethylene glycol on germination of lentil genotypes*. - Iranian J. Crop Res., 3(2): 91-101.

KAMALI M., SHOOR M., FEIZI H., 2018 - *Impacts of nanosized and bulk titanium dioxide on flowering and morphophysiological traits of petunia (*Petunia hybrida*) under salinity stress*. - J. Hort. Sci., 32: 199-212.

KARAMI A., SEPEHRI A., 2108 - *Nano titanium dioxide and nitric oxide alleviate salt induced changes in seedling growth, physiological and photosynthesis attributes of barley*. - Zemdirbyste Agric., 105: 123-132.

KHAN M.N., 2016 - *Nano-titanium Dioxide (Nano-TiO_2) mitigates NaCl stress by enhancing antioxidative enzymes and accumulation of compatible solutes in tomato (*Lycopersicon esculentum *Mill.)*. - J. Plant Sci., 11: 1-11.

KHOT L.R., SANKARAN S., MAJA J., EHSANI R., SCHUSTER E.W., 2012 - *Applications of nanomaterials in agricultural production and crop protection: A review*. - Crop Prot., 35: 64-70.

LYU S., WEI X., CHEN J., WANG C., WANG X., PAN D., 2017 - *Titanium as a beneficial element for crop production*. - Frontier in Plant Sci., 8: 1-19.

MAGUIRE I.D., 1982 - *Speed of germination - Aid in selection and evaluation for seedling emergence and vigor*. - Crop Sci., 22: 177-176.

MATTHEWS S., KHAJEH-HOSSEINI M., 2007 - *Length of the lag period of germination and metabolic repair explain vigor differences in seed lots of maize (*Zea mays*)*. - Seed Sci. Technol., 35: 200-212.

MIRI Y., MIRJALILI S.A., 2013 - *Effects of salinity stress on*

*seed germination and some physiological traits in primary stages of growth in purple coneflower (*Echinacea purpurea*)*. - Inter. J. Agron. Plant Prod., 4(1): 142-146.

MOHAMMADI N., GHASEMI GHAHSAREH M., BAHARVAND A., 2011 - *Study of salinity tolerance of seven ornamental plants in the germination stage of seed*. - Proc. 7th Iranian Congress of Horticultural Sciences, September 5-17, Isfahan. (In Persian).

MOTEVASEL N., ROOHI V., MOHAMMAD KHANI A., 2014 - *Effect of osmotic pretreatment on primrose seed germination at different levels of salinity stress*. - Proc. Second National Conference on Medicinal Plants and Sustainable Agriculture, September 22, Hamedan. (In Persian).

NASIR KHAN M., 2016 - *Nano-titanium dioxide (Nano-TiO_2) mitigates NaCl stress by enhancing antioxidative enzymes and accumulation of compatible solutes in tomato (*Lycopersicon esculentum *Mill*.). - J. Plant Sci., 11: 1-11.

NAVARRO E., BAUN A., BEHRA R., HARTMANN N.B., FILSER J., MIAO A., QUIGG A., SANTSCHI P.H., SIGG L., 2008 - *Environmental behavior and ecotoxicity of engineered nanoparticles to alga plants, and fungi*. - Ecotoxicology, 17: 372-386.

PARAVAR A., OMIDI H., 2104 - *Investigation of the effect of priming duration on germination characteristics of* Echinacea angustifolia *L. under salinity stress*. - Proc. First Inter. Congress and the 13th National Congress of Agronomy and Plant Breeding and the Third Conference on Seed Science and Technology, September 4-6, Karaj. (In Persian).

RAMAN P., PATINO L.C., NAIR M.G., 2004 - *Evaluation of metal and microbial contamination in botanical supplements*. - J. Agric. Food Chem., 52: 7822-7827.

RICHARDS L.A., 1954 - *Diagnosis and improvement of saline and alkali soils*. - Agriculture Handbook, USDA, Handbook No. 60

SANDRA C.M., 2004 - *Echinacea; The genus Echinacea*. - Med Arom. Plants-Indust. Pro., 39: 271.

STEPHANIE E.B., SVOBODA V.P., PAUL A.T., MARC W.V.I., 2005 - *Controlled drought affects morphology and anatomy of* Salvia solendens. - J. Amer. Soc. Hort. Sci., 130(5): 775-781.

SUN L.Z., CURRIER N.L., MILLER S.C., 1999 - *The American coneflower: a prophylactic role involving non specific immunity*. - J. Alternative Compl. Med., 5: 437-446.

TOKALLOO S., DAVOODI D., OMIDI M., EBRAHIMI M.A., ROOZBEH F., RASOOLNIA A., 2013 - *Effect of TiO_2 nanoparticles on germination traits and cytogenetic characteristics of barley*. - J. Agric. Biotech., 5(1): 13-25.

VASHISTH A., NAGARAJAN S., 2010 - *Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seed exposed to static magnetic field*. - J. Plant Physiol., 167: 149-156.

VIGOT E.L., ALMEIDA T.D., CHAGAS R.M., PONTE L.F.A., VIÉGAS R.A., SILVEIRA J.A.G., 2009 - *Source-sink regulation of cotyledonary reserve mobilization during cashew (*Anacardium occidentale*) seedling establishment under NaCl salinity*. - J. Plant Physiol., 166(1): 80-89.

YOUNES N.A., SHOKRY HASSAN H., ELKADY MARWA F., HAMED A.M., DAWOOD MONA F.A., 2020 - *Impact of synthesized metal oxide nanomaterials on seedlings production of three Solanaceae crops*. - Heliyon, 6. 03188.

ZHENG L., HONG F., LU S., LIU C., 2005 - *Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach*. - Biol. Trace Elem. Res., 105: 83-91.90

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Effect of a double phase culture system and activated charcoal on *in vitro* propagation of *Malus sylvestris* (L.) Mill.

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Abstract: The effectiveness of a double phase (solid/liquid) culture system (DPS) in comparison to a conventional (solid) system (CS) as well as, the role of various concentrations of activated charcoal in both systems on the enhancement of micropropagation of *Malus sylvestris* (L.) Mill. were investigated. In this study, lateral shoots were used as primary explants and a comparison for shoots regeneration and rooting abilities was assessed between DPS and CS micropropagation systems. Also, the effect of activated charcoal concentration (0, 250, 500, 1000 mg l^{-1}) during rooting stage was evaluated for both micropropagation systems. All assessed biometric parameters were higher in the DPS propagation system. The addition of activated charcoal induced effectively rhizogenesis in both systems, whereas the highest value of roots length (13.16 cm) was in the DPS system supplemented with activated charcoal at 500 mg l^{-1}. The DPS culture system represents a promising low-cost and time-saving technique which may improve micropropagation efficiency in producing a large quantity of homogenous wild apple plants.

1. Introduction

Malus sylvestris (L.) Mill. represents an autochthonous species of Albania. This distinct species is of great importance not only as a primary wild relative and potential gene donor for the domesticated apple (Stephan *et al*., 2003), but also for its medicinal values (Stojiljković *et al*., 2016). For years in Albania, no accurate inventory has been conducted for the vulnerable populations of this species which is classified in the category of threatened species, and maybe soon, it will be at possible risk of extinction (FAO, 2015; Gixhari and Ramadani, 2016). In Europe also, there is a lack of information regarding its geographical distribution and for this reason, in The IUCN Red List of Threatened Species (IUCN, 2019), it is

included in the category of 'data deficient' species. Beside this, it is also reported as endangered in some European countries (Larsen *et al*., 2006; Wagner *et al*., 2014). The specific need for agricultural yield is one of the key reasons for the development of tissue culture technology worldwide. Indeed, plant micropropagation is an efficient method of propagating disease-free, genetically uniform and massive amounts of plants under *in vitro* conditions (Gupta *et al.,* 2020). Over the years, the number of plant species which have been clonally propagated through tissue culture was increased and the most commercially important species have been studied. *In vitro* propagation techniques have found wide use because of their effectiveness in terms of the high-quality product obtained and of reduced cost (Jain and Ishii, 2003; Debnath *et al*., 2006; Damiano *et al*., 2008; Lambardi *et al*., 2013). Clonal propagation creates the possibility of obtaining a large quantity of homogeneous plant material, which can be conserved for short/mid-term periods through minimal growth methods, or long-term period, through cryopreservation (Kameswara, 2004; Day and Stacey, 2007; Benelli *et al*., 2012). For all these advantages, and also because of the current situation on the geographical distribution and importance of *M. sylvestris*, it is of strategic importance to develop an effective micropropagation protocol, to obtain significant numbers of clonal plantlets which can be used for *ex situ* conservation strategies or other purposes.

In all micropropagation methods, the main goal is to optimize a successful protocol that ensures a rapid clonal propagation and results also as a time-saving technique (Lambardi *et al*., 2013). In most reports, the protocols implemented for *Malus* sp. micropropagation are based on conventional micropropagation systems in semisolid culture media which typically include explants inoculation/proliferation, subculture, and rooting steps. Several authors mentioned the effective stabilization of wild apple micropropagation using conventional micropropagation system consisting in a monophasic/agarized medium (Modgil *et al*., 1999; Boudabous *et al*., 2010; Dobránszki *et al*., 2011; Kereša *et al*., 2012; Zhang *et al*., 2020). But Teixeira da Silva *et al*. (2019), in a review regarding tissue culture of *Malus* sp., mentioned that most reports aimed to find alternative gelling agents other than agar, in order to reduce costs.

Although agarized media are successfully used for plant micropropagation, nowadays it has become absolutely important to improve the productivity and uniformity of valuable vegetal materials with economic values by reducing the cost of production, space, time or, optimizing other issues related to micropropagation coefficient, rooting index, etc. In addition to solid media, several techniques have been successfully practiced for the micropropagation of economically important plants such as 1) the use of liquid cultures for the micropropagation of two apple rootstocks (Mehta *et al*., 2014), pineapple (Dal Vesco *et al*., 2001) and *Dioscorea* sp. (Jova *et al*., 2011); 2) the use of continuous immersion bioreactors for apple rootstock (Chakrabarty *et al*., 2003), eucalyptus (Mendonça *et al*., 2016), chestnut (Vidal *et al*., 2017), hybrid chestnut (Cuenca *et al*., 2017); 3) the use of temporary immersion bioreactors for wild apple (Sota *et al*., 2021), apple rootstocks (Chakrabarty *et al*., 2003; Zhu *et al*., 2005), and oak (Gatti *et al*., 2017).

An alternative for improving *in vitro* micropropagation protocols is the use of double-phase nutrient media (DPS). In this method, the explants are fixed in a solid medium, while the liquid medium is periodically added during the culture, therefore eliminating the need for subcultures. In this way, the propagation costs and the chances of contamination are reduced (Senapati, 2015). There are very few reports on the use of double-phase media for *in vitro* propagation of plants (Scherwinski-Pereira *et al*., 2012; Lopez and Suarez, 2018). In most reports, the same culture medium was used in solid and liquid phases (except agar presence) to increase the plantlets' mass production during the cultures. But it would also be useful and interesting to test various media for propagation and rooting in the same culture container. In this case, the solid phase would have a hormonal content effective for rooting induction, while the liquid one would have a hormonal content to induce lateral shoots development. Furthermore, the addition of activated charcoal (AC) in the culture media enhances *in vitro* rooting induction/development in some fruit-trees species (Wang and Huang, 1976; Thomas, 2008), and also acts as an adsorbent of phenolic compounds to avoid oxidation phenomena (Boudabous *et al*., 2010; Shinde *et al*., 2010).

This study aimed to evaluate the efficiency of DPS (solid/liquid culture system) in comparison with the conventional CS (solid) propagation system for improving *in vitro* shoots regeneration of *Malus sylvestris* (L) Mill. In addition, the effect of various concentrations of AC in both systems on rooting abilities was evaluated.

2. Materials and Methods

Plant material and micropropagation systems

Axillary buds of wild apple [*M. sylvestris* (L.) Mill.] were excised from scions of the population of Maminas at Durrës County in western Albania and were used as initial explants. The explants were disinfected with 70% ethanol for 3 min, followed by the treatments with 0.2% of 50% carbendazim (Bavistine) for 7 min and 0.01% $HgCl_2$ for 10 min., and multiple rinses with sterile distilled water were performed.

In this research, two micropropagation systems for *in vitro* regeneration of *M. sylvestris* plantlets were compared:

- *Conventional micropropagation system* (CS), consisting in the explants culture on solid medium (monophase system);
- *Double phase system* (DPS), consisting in the explants culture on solid medium plus a liquid phase at the same time.

For DPS micropropagation systems, the liquid medium was added every week in the culture vessels, specifically 1 ml in the test tubes (proliferation stage) and 3 ml in Erlenmeyer flasks (rooting stage). All procedures were performed under aseptic conditions.

Media composition and culture conditions

In vitro shoots proliferation. MS medium (Murashige and Skoog, 1962) was used, supplemented with 1 mg l^{-1} 6-benzylaminopurine (BAP) and 0.1 mg l^{-1} α-naphtalenacetic acid (NAA) for both liquid and solid phase.

Rhizogenesis induction. The liquid phase was the same as in the proliferation stage, while for both systems under study the solid medium was supplemented with 1 mg l^{-1} NAA and different concentrations (0, 250, 500, 1000 mg l^{-1}) of activated charcoal (AC).

In all cases, in the medium was added sucrose at 3%, while, for solid medium preparation, 7 g l^{-1} of agar (Sigma-Aldrich) was also supplemented. The pH was adjusted to 5.7 before medium autoclaving at 120°C for 20 min.

The cultures were maintained in the growth chamber at 25±2°C in a 16 h/8 h light/dark regime with cool, white fluorescent light of intensity 43.4 μmol m^{-2} s^{-1}.

Data elaboration

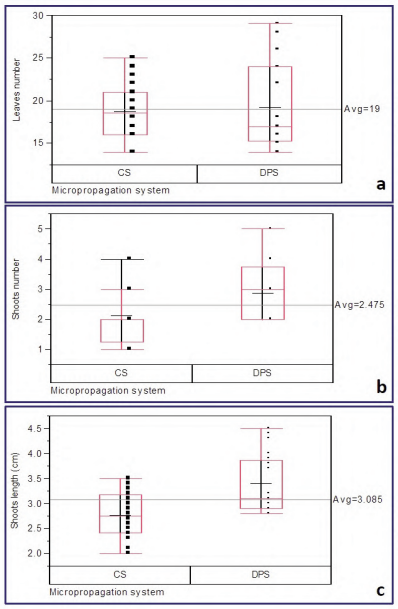
Leaves number, shoots number, shoots length, roots number and roots length, were evaluated after 42 days of culture for each micropropagation stage. Experimental data were elaborated by Tukey-Kramer test, and the analysis of variance (ANOVA) with JMP 7.0 statistical software.

3. Results and Discussion

In vitro regeneration wild apple shoots in the DPS and CS systems

After 42 days of *in vitro* culture, the growth dynamic of wild apple explants in the CS and DPS micropropagation systems was evaluated. During proliferation, for both systems, the PGRs ratio was such that induced lateral shoots regeneration. The comparative growth dynamic between the two propagation systems for leaves number, shoots number and shoots length, is presented in the variability charts (Fig. 1), and it clearly shows that the micropropagation system highly affected growth parameters (Fig. 2 a-e). The contact of the explants with the liquid medium (Fig. 2 c), facilitated and increased the amount of nutrients absorbed by the explants while in the solid medium (Fig. 2 a; b), the solid consistency itself slowed down the rate of absorption.

In our findings, the number of leaves for shoot in both systems, was high, specifically 18.75 during the culture in the CS system, and 19.25 in the DPS system (Fig. 1 a).



	Microprop. system	Mean ± Std. error
Leaves number	CS	18.75 ± 0.70
	DPS	19.25 ± 1.09

	Microprop. system	Mean ± Std. error
Shoots number	CS	2.10 ± 0.22
	DPS	2.85 ± 0.20

	Microprop. system	Mean ± Std. error
Shoots length	CS	2.77 ± 0.09
	DPS	3.39 ± 0.13

Fig. 1 - Variability chart: leaves number (a); shoots number (b); shoots length (cm) (c), in the DPS and CS micropropagation systems.

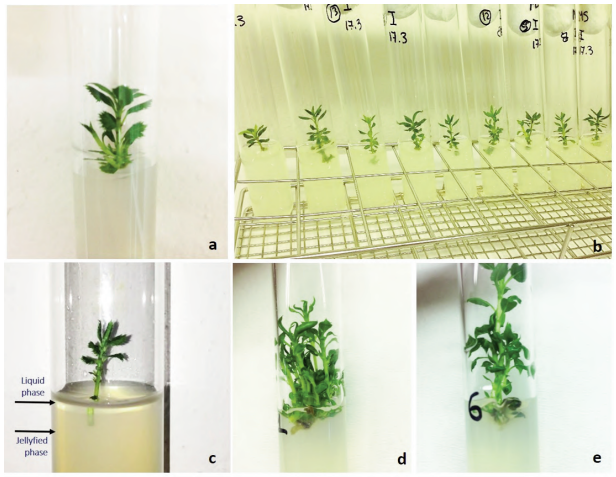


Fig. 2 - *In vitro* regeneration of wild apple shoots in CS micropropagation system (a, b) and in DPS micropropagation system (c, d, e).

DPS showed a higher efficiency for shoots number and shoot length, 2.85 and 3.39 cm, respectively, compared to CS, with 2.10 shoots number and 2.77 cm shoots length (Fig. 1 b; c). Overall, the explants grown in the CS propagation system, showed a lower regeneration potential for the monitored biometric parameters, which can be related to the absorption of substances from the nutrient medium. The greater thickness of the shoots cultivated in the DPS system was evident during the proliferation phase (Fig. 2 d; e).

The efficiency of the DPS system due to the presence of the liquid phase that enhanced the contact area of the explant with the nutrient medium, leading to an increase in the rate of diffusion, absorption and, continuous replacement of nutrients consumed over the days of culture is reported by various authors stressing the efficiency of the double phase micropropagation system (Moraes *et al*., 2004; Pullman and Skryabina, 2007; Scherwinski-Pereira *et al*., 2012; Dorić *et al*., 2014; Senapati, 2015). In this regard, Rodriguez *et al*. (1991) found that the micropropagation rate of *Pyrus communis* L., especially in terms of axillary shoots formation, was higher when a liquid medium was added onto the jellified one. The superiority of DPS compared with CS system is also reported by Oliveira *et al*. (2013) on *in vitro* propagation of vanilla. In this species, axillary shoot multiplication was greatest in DPS, with an increase over 2.5-fold in comparison to the solid medium system, after 90 days of cultivation. Similarly, Couselo *et al*. (2006) noted that the micropropagation rates of Albariño plants were significantly higher when culturing in a DPS system with the same concentration of BA (8.88 µM) in both phases, in comparison to a monophasic one. The same trend on the efficiency of DPS cultivation system was reported for the micropropagation of arrow cane (Lopez and Suarez, 2018) ananas (Scherwinski-Pereira *et al*., 2012), *Pyrus* sp. (Moraes *et al*., 2004), and *Rauwolifa serpentine* (Senapati, 2015). On the contrary, Barceló-Muñoz *et al*. (1999) during micropropagation of avocado, reported that continuous culturing under DPS conditions induced succulence in shoot bases and hyperhydric of the cultures.

Together with the effectiveness for the growth dynamic, DPS has another advantage due to the periodic addition of the liquid medium. In this condition, the period from one subculture to the other can be longer than in a monophasic liquid or solid medium, where this period is up to 3-4 weeks, as reported also by Mahmad *et al*. (2014).

The CS system needs to change the medium in culture vessels after some time to avoid nutrient deficiencies. All this requires a series of laborious operations in terms of costs, both of hand labor and chemicals. Moreover, there is more working time in the cabinet laminar flow which creates possibilities for increasing the percentage of culture contamination. So, it can be said that in DPS propagation system, the cost of plant production is reduced. This finding is also accurately reported by Lopez and Suarez (2018) who calculated the costs of production per plant and found that, for arrow cane, the cultivation in the DPS system reduced the micropropagation costs by 20%. A similar estimation in terms of production cost was also realized by Senapati (2015) who found that DPS system was much more effective due to the costs reduction specifically with 33.36% on the nutrients used, 39.28% on the energy used and 33.33% on the labor costs. Optimization of such technique that, in addition to being low cost, also provides rapid *in vitro* plantlets regeneration, is of great interest for commercial use.

Biomass production during the rooting stage

After the proliferation stage, the shoots were transferred in DPS system and CS system for rooting. The first system had in the liquid phase the same type and concentration of PGRs of the proliferation stage, to promote the shoots development and at the same time, the solid phase was prepared to give

a rhizogenic induction. The medium of CS system was supplemented with rooting induction hormone (IAA). The biometric parameters were greatly affected, during this stage, not only by the propagation system but also by the concentration of activated charcoal in the media (Table 1).

not show significant differences between DPS and CS. This indicated that AC, with concentrations > than 250 mg l^{-1}, was the determining factor to improve rooting response. In this propagation stage, also DPS system resulted most advantaged in comparison to CS one.

Table 1 - The effect of the micropropagation system and activated charcoal concentration on roots and shoots development during the rooting phase of *M. sylvestris*

Propagation system	Activated charcoal (mg l^{-1})	Roots		Shoots	
		length (cm)	number	length (cm)	number
DPS	0	2.46 ± 0.16 e	2.66 ± 0.21 e	3.40 ± 0.15 c	3.53 ± 0.38 e
	250	5.23 ± 0.27 d	4.86 ± 0.31 d	4.61 ± 0.62 b	5.61 ± 0.36 b
	500	13.16 ± 0.67 a	8.73 ± 0.51 b	5.58 ± 0.17 a	7.46 ± 0.48 a
	1000	11.03 ± 0.30 b	9.80 ± 0.29 a	4.76 ± 0.17 b	5.40 ± 0.32 bc
CS	0	2.22 ± 0.11 e	2.46 ± 0.24 e	2.74 ± 0.11 d	2.40 ± 0.19 f
	250	4.81 ± 0.22 d	4.33 ± 0.28 d	3.18 ± 0.14 c	3.93 ± 0.31 de
	500	9.93 ± 0.33 c	7.33 ± 0.39 c	3.37 ± 0.12 c	4.60 ± 0.25 cd
	1000	10.40 ± 0.39 bc	7.53 ± 0.29 c	3.02 ± 0.12 cd	4.26 ± 0.30 de

Data represents mean ± standard error. Within each column, data followed by different letters are significantly different at P≤0.05 by Tukey-Kramer test.

Rhizogenesis induction

From a comparison between the two propagation systems for the same concentration of AC, in general, the best result was achieved in the DPS propagation system. Overall, rooting induction was highly affected by the concentration of AC in the culture media, and it was observed a positive correlation between the concentration of AC in the media and the rhizogenesis rate (Table 1; Fig. 3 a, b).

For roots length parameter, during the DPS culture, the best result (13.16 cm) was obtained at 500 mg l^{-1} of AC concentration; while the higher roots number (9.80) was showed at 1000 mg l^{-1} of AC concentration. In the CS, for both parameters, the best results were achieved at 1000 mg l^{-1} of AC, respectively with 10.40 cm for roots length and 7.53 for roots number. There were no significant differences for the roots number parameter in CS propagation system between culture media supplemented with 1000 or 500 mg l^{-1} of AC.

From an overall evaluation and comparison for the rooting response depending on both propagation system and AC concentration, the optimal condition for *in vitro* rooting of wild apple shoots was on DPS propagation system supplemented with 1000 mg l^{-1} or 500 mg l^{-1} of AC.

On culture medium supplemented with 250 mg l^{-1} of AC or AC-free medium, the rooting response did

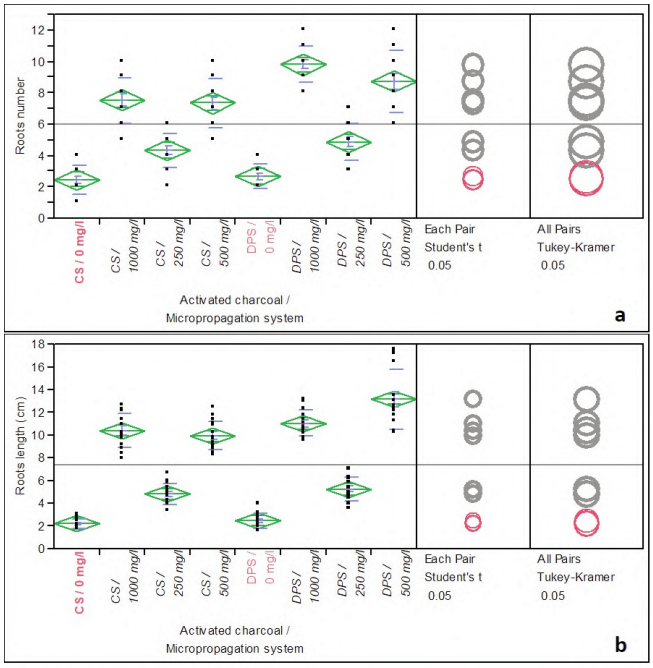


Fig. 3 - One-Way ANOVA analysis of roots number (a) and roots length (cm) (b) depending on the activated charcoal (AC) concentration and micropropagation system.

New shoots regeneration during the rooting stage

Since the liquid phase in the DPS had a hormonal ratio improving the development of lateral buds, at this stage also the response of plants in terms of mass formation of new shoots was evaluated. The

solid phase in both CS and DPS was optimized only for the induction of rhizogenesis.

As it can be seen from data presented (Table 1; Fig. 4 a, b and Fig. 5) the differences regarding shoots length and number between DPS and CS cultivation systems are highly significant.

The comparison between the DPS and CS propagation systems showed the considerable DPS efficiency for shoots length and shoots number. In particular, in AC-free media, the differences were highly significant, 3.40 cm (shoots length) and 3.53 (shoots number) for DPS in comparison with 2.74 cm and 2.40 shoots, respectively, in the CS. Obviously, DPS was more effective due to the supplementation of liquid phase with hormones responsible for shoots proliferation. Also, this proved further that the double phase system was effective for the growth of plantlets and at the same time for their rooting. During the rooting stage, the DPS allowed the simultaneous formation of roots and new shoots at a higher rate, thus leading to the possibility to reduce the micropropagation cost by combining in a single one the last stage of subculture and rooting.

The presence of AC supported also the development of additional shoots, in both propagation systems, but in DPS this effect was more pronounced and significant in comparison to CS. Moreover, the AC influence increased until 500 mg l^{-1} concentration, and, indeed at this AC concentration, the DPS

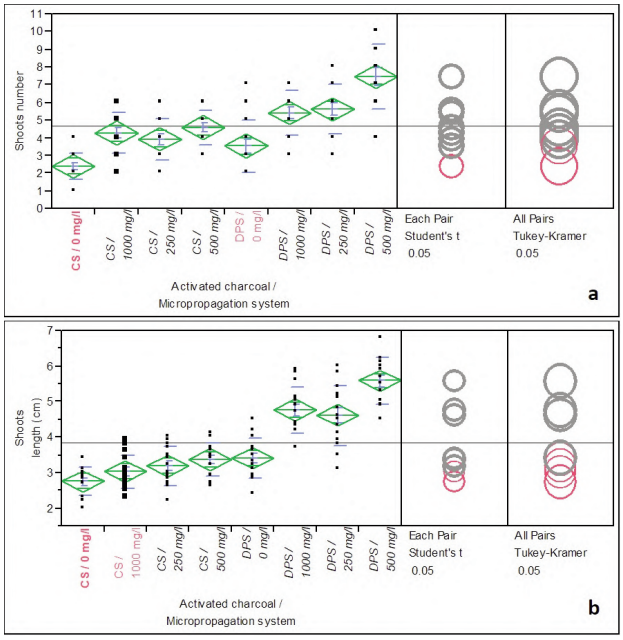


Fig. 4 - One-Way ANOVA analysis of shoots number (a) shoots length (cm) (b) depending on the activated charcoal (AC) concentration and micropropagation system.

Fig. 5 - Rhizogenesis induction and mass plantlets production during the rooting stage: culture in AC-free media (a); Wild apple cultures grown in various concentrations of AC (b); roots measurements (c); mass production of rooted plantlets (d).

showed the higher rate of shoots number (7.46) and shoots length (5.58 cm). The same tendency was observed even in CS where the concentration of AC at 500 mg l^{-1} gave higher efficiency, with 3.37 cm of the shoots length and 4.60 for the shoots number.

The results indicated that AC concentration in the media was an important factor that highly affected micropropagation rate. Roots and new shoots formation responses were positively correlated and were dependent on the AC concentration in the nutrient media. In AC-free media, the roots formation was not at a high rate in comparison to the other AC concentrations.

Addition of activated charcoal in culture medium may affect *in vitro* plants growth, in terms of multiplication ratio, shoot elongation, rooting and embryogenesis (Pan and Van Staden, 2001; Thomas, 2008; Abdulwahed, 2013). Most publications have focused on the effects of activated charcoal on tissue response during *in vitro* propagation, and it was shown that its use may either promote or inhibit growth under *in vitro* conditions, depending on different factors. Boudabous *et al*. (2010) reported that the use of MS-half medium supplemented with 200 mg l^{-1} of AC and 3.0 mg l^{-1} of IBA, was highly effective on *in vitro* rhizogenesis of apple. On the other hand, Magyar-Tábori *et al*. (2002), in their study didn't find any favourable effect of activated charcoal on rooting characteristics of apple, but the plants originated from media that contained activated charcoal grew more vigorously during rooting and acclimatization.

In cotton (*Gossypium hirsutum*), the addition of AC in the medium enhanced shoots and roots induc-

tion as well as shoots length from split embryo axes as compared to MS basal medium (Hazra *et al*., 2002). Also, Dev *et al*. (2015) mentioned that the use of 200 mg l^{-1} of AC, significantly improved *in vitro* multiplication of some grape genotypes, meanwhile Hassan *et al*. (2011) found that the presence of AC in the medium enhanced microtuberization and *in vitro* regeneration of potato plantlets. Moreover, it is widely accepted that some of the beneficial effects of activated charcoal can be attributed to the removal of inhibitory substances from the nutrient medium. This phenomenon is mostly considerable for *M. sylvestris in vitro* propagation, because shoot explants of this plant species even after establishment and several subcultures shows browning at the shoot base (Sota *et al*., 2021).

Roots grow according to negative phototropism and, in many cases, light is considered as a stress factor for roots induction (Silva-Navas *et al*., 2015) and it is reported that the combination of darkness and exogenous auxins enhance rooting response (Monteuuis and Bon, 2000; López-Pérez and Martínez, 2015). In our research, the presence of AC in the nutrient medium created a state of darkness, thus enabling the formation of a well-developed roots system. This ensured a higher absorption of the nutrients, which can lead also to new shoots formation and development. Furthermore, the presence of a higher concentration of cytokinins *vs.* auxins in the liquid phase also was responsible for lateral shoots development. In this context, in our study, both the propagation system types and the presence of AC in the media, highly affected the micropropagation rate and the quality of regenerated plantlets.

Moreover, another advantage of DPS in comparison to CS system that can explain enhanced growth is the weekly addition of the liquid media into the vessel during the culture period, since the plantlets did not suffer mineral deficiencies.

4. Conclusions

Malus sylvestris, wild apple*,* is a very important plant species and is properly considered a threatened one; in such situation, the constant optimization of the micropropagation protocols is a necessity. The specific type of propagation system plays a key role in enhancing growth parameters. In the present study, our findings sufficiently indicated that the use of the DPS propagation system in comparison to CS one was highly effective during *in vitro* shoots' regeneration and rooting stage. Moreover, the addition of activated charcoal for rooting induction obviously improved not only roots formation but also the growth of wild apple shoots. Among various concentrations of AC examined, the addition of 500 mg l^{-1} AC in culture medium was found to be the best concentration for this process. The obtained results demonstrated a direct correlation between the propagation system used and the concentration of AC in the culture media. The application of DPS propagation system for *in vitro* propagation of wild apple, therefore represents more time-saving and a low-cost technique in comparison to the CS. These findings could provide a platform for progressively improving the clonal propagation of wild apple plantlets grown under *in vitro* conditions, leading so to the possibility for the effective use of these plantlets for its *ex situ* conservation or other scientific or practical purposes.

References

ABDULWAHED M.S., 2013 - *Identification of the effect of different levels of activated charcoal and sucrose on multiplication shoots of date palm* Phenix dactylifera *L. cv. Sufedy* in vitro. - J. Hortic. Forestry, 5(9): 139-145.

BARCELÓ-MUÑOZ A., ENCINA C.L., SIMÓN-PÉREZ E., PLIEGO-ALFARO F., 1999 - *Micropropagation of adult avocado.* - Plant Cell Tiss. Organ Cult., 58: 11-17.

BENELLI C., OZUDOGRU E.A., LAMBARDI M., DRADI G., 2012 - In vitro *conservation of ornamental plants by slow growth storage.* - Acta Horticulturae, 961: 89-93.

BOUDABOUS M., MARS M., MARZOUGUI N., FERCHICHI A., 2010 - *Micropropagation of apple* (Malus domestica *L. cultivar Douce de Djerba) through* in vitro *culture of axillary buds.* - Acta Bot. Gallica, 157: 513-524.

CHAKRABARTY D., HAHN E.J., YOON Y.J., PAEK K.Y., 2003 - *Micropropagation of apple rootstock M.9 EMLA using bioreactor*. - J. Hort. Sci. Biotech., 78(5): 605-609.

COUSELO J.L., VARELA P., REY M., 2006 - *Effect of benzyladenine concentration and double-phase culture system on* in vitro *multiplication of adult* Albari *plants.* - Amer. J. Enol. Vitic., 57: 109-112.

CUENCA B., SÁNCHEZ C., ALDREY A., BOGO B., BLANCO B., CORREA B., VIDAL N., 2017 - *Micropropagation of axillary shoots of hybrid chestnut* (Castanea sativa x C. crenata) *in liquid medium in a continuous immersion system.* - Plant Cell Tiss. Organ Cult., 131: 307-320.

DAL VESCO L.L., DE ALMEIDA PINTO A., ZAFFARI G.R., NODARI R.O., DOS REIS M.S., GUERRA M.P., 2001 - *Improving pineapple micropropagation protocol through explant size and medium composition manipulation.* - Fruits, 56(3): 143-154.

DAMIANO C., ARIAS PADRO M.D., FRATTARELLI A., 2008 - *Propagation and establishment* in vitro *of myrtle (*Myrtus communis *L.), pomegranate (*Punica granatum *L.) and mulberry (*Morus alba *L.).* - Propag. Ornam. - Plants, 8(1): 3-8.

DAY J.G., STACEY G.N., 2007 - *Cryopreservation and freeze-drying protocols*. - Second Edition. Humana Press, Totowa, New Jersey, USA, pp. 365.

DEBNATH M., MALIK C.P., BISEN P.S., 2006 - *Micropropagation: A tool for the production of high quality plant-based medicines.* - Curr. Pharmac. Biotechn., 7(1): 33-49.

DEV R., SINGH S.K., SINGH A.K., VERMA M.K., 2015 - *Comparative* in vitro *multiplication of some grape* (Vitis vinifera) *genotypes.* - Ind. J. Agr. Sci., 85: 1477-1483.

DOBRÁNSZKI J., MAGYAR-TÁBORI K., TOMBÁCZ E., 2011 - *Comparison of the rheological and diffusion properties of some gelling agents and blends and their effects on shoot multiplication.* - Plant Biotechnol. Rep., 5: 345-352.

DORIĆ D., OGNJANOV V., LJUBOJEVIĆ M., BARAĆ B., DULIĆ J., PRANJIĆ A., DUGALIĆ K., 2014 - *Rapid propagation of sweet and sour cherry rootstocks.* - Not. Bot. Horti. Agrobo., 42(2): 488-494.

FAO, 2015 - *Global forest resources assessment 2015*. - Food and Agriculture Organization of the United Nations, Rome, Itay, pp. 245.

GATTI E., SGARBI E., OZUDOGRU E.A., LAMBARDI M., 2017 - *The effect of Plantform™ bioreactor on micropropagation of* Quercus robur *in comparison to a conventional* in vitro *culture system on gelled medium, and assessment of the microenvironment influence on leaf structure.* - Plant Biosyst., 151: 1129-1136.

GIXHARI B., RAMADANI M., 2016 - *Agrobiodiversity in Fushë Arrëz.* - Monograph published within the project founded by UN "Increasing the capacity of the community of Fushë-Arrëz for the preservation of autochthonous agricultural, nut and fruit seeds". (In Albanian).

GUPTA N., JAIN V., JOSEPH M., DEVI S., 2020 - *A review on micropropagation culture method*. - Asian J. Pharm., 8: 86-93.

HASSAN M.L., BEHROOZ E., ESMAIE C., 2011 - *Hinokitiol and activated charcoal influence the microtuberization and growth of potato (*Solanum tuberasum *cv. Agria) plantlets* in vitro. - Australian J. Crop Sci., 5(11): 1481-1485.

HAZRA S., KULKARNI A.V., BANERJEEE A.K., DHAGE A.B., AGRAWAL D.C., KRISHNAMURTHY K.V., NALAWADE S.M., 2002 - *A rapid and simple method for* in vitro *plant regeneration from split embryo axes of six cultivars of cotton.* - Biologia Plantarum, 45(2): 317:319.

IUCN, 2019 - *The IUCN red list of threatened species.* - https://www.iucnredlist.org.

JAIN S.M., ISHII K., 2003 - *Micropropagation of woody trees and fruits.* - Springer, Dordrecht, The Netherlands, pp. 840.

JOVA M.C., KOSKY R.G., CUELLAR E.E., 2011 - *Effect of liquid media culture systems on yam plant growth (*Dioscorea alata *L. 'Pacala Duclos').* - Biotechnol. Agron. Soc. Environ., 15(4): 515-521.

KAMESWARA N.R., 2004 - *Plant genetic resources: Advancing conservation and use through biotechnology.* - African J. Biotechn., 3(2): 136-145.

KEREŠA S., MIHOVILOVIĆ BOŠNJAK A., BARIĆ M., HABUŠ JERČIĆ I., ŠARČEVIĆ H., BIŠKO A., 2012 - *Efficient axillary shoot proliferation and* in vitro *rooting of apple cv. 'Topaz'.* - Notulae Botanica Horti Agrobotanici, 40(1): 113-118.

LAMBARDI M., OZUDOGRU E.A., JAIN S.M., 2013 - *Protocols for micropropagation of selected economically-important horticultural plants. Methods in Molecular Biology Series*. - Springer Science, New York, USA, pp. 490.

LARSEN A.S., ASMUSSEN C.B., COART E., OLRIK D.C., KJAER E.D., 2006 - *Hybridization and genetic variation in Danish populations of European crab apple* (Malus sylvestris). - Tree Genetics & Genomes, 2(2): 86-97.

LOPEZ C., SUAREZ I., 2018 - In vitro *arrow cane (*Gynerium sagitatum *Aubl.) multiplication in double phase médium.* - Revista de Ciencias Agrícolas, 35(2): 5-13.

LÓPEZ-PÉREZ A.J., MARTÍNEZ J.A., 2015 - In vitro *root induction improvement by culture in darkness for different globe artichoke cultivars.* - In Vitro Cell. Dev. Biol. - Plant, 51: 160-165.

MAGYAR-TÁBORI K., DOBRÁNSZKY J., JÁMBOR-BENCZÚR E., LAZÁNYI J., SZALAI J., FERENCZY A., 2002 - *Effects of indole-3-butyric acid levels and activated charcoal on rooting of* in vitro *shoots of apple rootstocks.* - Inter. J. Hortic. Sci., 8: 3-4.

MAHMAD N., TAHA R.M., OTHMAN R., SALEH A., HASBULLAH N.A., ELIAS H., 2014 - *Effects of NAA and BAP, double-layered media, and light distance on* in vitro *regeneration of* Nelumbo nucifera Gaertn. *(Lotus), an aquatic edible plant.* - The Scientific World Journal, pp. 1-8.

MEHTA M., RAM R., BHATTACHARYA A., 2014 - *A simple and cost effective liquid culture system for the micropropagation of two commercially important apple rootstocks.* - Indian J. Exper. Biology, 52: 748-754.

MENDONÇA E.G., STEIN V.C., CARVALHO H.H., DE SANTOS B.R., BEIJO L.A., PAIVA L.V., 2016 - *The use of continuous, temporary immersion bioreactor system and semisolid culture medium for the production of* Eucalyptus camaldulensis *clones.* - Ciência Florestal, 26: 1211-1224.

MODGIL M., SHARMA D.R., BHARDWAJ S.V., 1999 - *Micropropagation of apple cv.* 'Tydeman's' 'Early Worcester'. - Scientia Hortic., 81: 179-188.

MONTEUUIS O., BON M.C., 2000 - *Influence of auxins and darkness on in vitro rooting of micropropagated shoots from mature and juvenile* Acacia mangium. - Plant Cell, Tiss. Organ Cult., 63: 173-177.

MORAES L., FELISBINO C., CRESTANI L., SILVA A., 2004 - In vitro *establishment and multiplication of* Pyrus calleryana *D-6 on double-phase culture system.* - Rev. Brasileira de Fruticultura, 26(3): 403-405.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tabacco cultures.* - Physiologia Plantarum, 15: 473-497.

OLIVEIRA S.O.D., SAYDB R.M., BALZONC T.A., SCHERWINSKI-PEREIRA J.E., 2013 - *A new procedure for* in vitro *propagation of vanilla* (Vanilla planifolia) *using a double-phase culture system*. - Scientia Horticulturae, 161: 204-209.

PAN M., VAN STADEN J., 2001 - *The effect of activated charcoal on the production and development of somatic embryos in cultures of carrot* Daucus carota. - South African Journal of Botany, 67: 629-635.

PULLMAN G.S., SKRYABINA A., 2007 - *Liquid medium and liquid overlays improve embryogenic tissue initiation in conifers*. - Plant Cell Rep., 26(7): 873-887.

RODRIGUEZ R., DIAZ-SALA C., CUOZZO L., ANCORA G., 1991 - *Pear* in vitro *propagation using a double-phase culture system.* - HortScience, 26(1): 62-64.

SCHERWINSKI-PEREIRA J.E., LIMA E.C.A., DA SILVA T.L., MESQUITA A.G.G., MACIEL S.A., COSTA F.H.S., 2012 - *Double-phase culture system for large scale production of pineapple*. - Plant Cell, Tiss. Organ Cul., 109(2): 263-269.

SENAPATI S.K., 2015 - *A double phase culture system: an economic and time saving protocol for* in vitro *propagation of plant.* - SAJ Biotechnol., 2: 101.

SHINDE K.A., PATEL R.M., SHAH R.R., 2010 - *Proliferation, rooting and acclimatization of micropropagated grape* cv. Thompson Seedless. - Int. J. Plant Sci., 5: 98-101.

SILVA-NAVAS J., MORENO-RISUENO M.A., MANZANO C., PALLERO-BAENA M., NAVARRO-NEILA S., ET AL. 2015 - *D-Root: a system for cultivating plants with the roots in darkness or under different light conditions*. - The Plant Journal, 84: 244-255.

SOTA V., BENELLI C., ÇUKO B., PAPAKOSTA E., DEPAOLI C., LAMBARDI M., KONGJIKA E., 2021 - *Evaluation of ElecTIS bioreactor for the micropropagation of* Malus sylvestris *(L.) Mill., an important autochthonous species of Albania.* - Hort. Sci. (Prague), 48: 12-21.

STEPHAN B.R., WAGNER I., KLEINSCHMIT J., 2003 - *Wild apple and pear - Malus sylvestris/Pyrus pyraster: Technical guidelines of genetic conservation and use* (PDF) - European Forest Genetic Resources Programme.

STOJILJKOVIĆ D., ARSIĆ I., TADIĆ V., 2016 - *Extracts of wild apple fruit (*Malus sylvestris *(L.) Mill., Rosaceae) as a source of antioxidant substances for use in production of nutraceuticals and cosmeceuticals*. - Industrial Crops and Products, 80: 165-176.

TEIXEIRA DA SILVA J.A., GULYÁS A., MAGYAR-TÁBORI K., WANG M.R., WANG Q.C., DOBRÁNSZKI J., 2019 - In vitro *tissue culture of apple and other* Malus *species: recent advances and applications.* - Planta, 249: 975-1006.

THOMAS D., 2008 - *The role of activated charcoal in plant tissue culture*. - Biotechnology Advances, 26(6): 618-631.

VIDAL N., ALDREY A., BLANCO B., CORREA B., SÁNCHEZ C., CUENCA B., 2017 - *Proliferation and rooting of chestnut under photoautotrophic conditions*. - In: BONGA J.M., Y.S. PARK, and J.F. TRONTIN (eds.). Proceedings of the 4th International Conference of the IUFRO Unit 2.09.02 on "*Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment*". La Plata, Argentina, pp. 119-127.

WAGNER I., MAURER W.D., LEMMEN P., SCHMITT H.P., WAGNER M., BINDER M., PATZAK P., 2014 - *Hybridization and genetic diversity in wild apple (*Malus sylvestris *L. Mill) from various regions in Germany and from Luxembourg*. - Silvae Genetica, 63(3): 81-94.

WANG P., HUANG L., 1976 - *Beneficial effects of activated charcoal on plant tissue and organ cultures.* - In Vitro, 12(3): 260-262.

ZHANG Y., BOZOROV T.A., LI D.X., ZHOU P., WEN X.J., DING Y., ZHANG D.Y., 2020 - *An efficient* in vitro *regeneration system from different wild apple* (Malus sieversii) *explants.* - Plant Methods, 16(56): 1-10.

ZHU L.H., LI X.Y., WELANDER M., 2005 - *Optimisation of growing conditions for the apple rootstock M26 grown in RITA® containers using temporary immersion principle.* - Plant Cell Tiss. Organ Cult., 81: 313-318.

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Histological and physiological changes of potato starch derived from seed and TPS (True Potato Seed) grown tubers under different cold storage duration

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Abstract: Starch granules in potato tubers exist with varying sizes and size distribution in nature. In this study, both the tubers of seed potato ('Lady Rosetta') and true potato seed (TPS) ('BARI TPS-1') varieties were stored at 5°C for 0 to 4 months, and the changes in the starch break down were analyzed physiologically and histologically to investigate how cold storage affects the starch break down. Although the starch content of both varieties reduced during cold storage, the reduction of starch content in 'BARI TPS-1' was higher than that of 'Lady Rosetta'. However, both volume and ovality (length:width) of starch granule did not change significantly throughout the storage period irrespective of variety, suggesting a non-uniform breakdown of starch granules. Scanning Electron Microscope (SEM) images of starch granule showed non-uniformed deformation and enlarged cavity or hole along the storage period, which indicated that starch breakdown occurred at a specific part of starch granule rather than peripherally and penetration would be deeper in 'BARI TPS-1' than that of 'Lady Rosetta'. However, there was no significant change in granule size distribution in spite of rapid degradation of amylopectin percentage in 'BARI TPS-1' than that of 'Lady Rosetta', suggesting more susceptibility of 'BARI TPS-1' to starch degrading enzyme and higher enzymatic action would cause deeper penetration in 'BARI TPS-1' than that of 'Lady Rosetta'.

1. Introduction

Potato (*Solanum tuberosum* L.) crop is usually cultivated by planting seed tubers which are genetically identical clones. On the other hand, True Potato Seed (TPS) is the actual botanical potato seed produced by the potato plant. Potato production from seed tuber derived

from TPS (seedling tuber) is emerging as a promising alternative of using seed tuber due to the advantages of less disease transmission, physiological maturity until planting season, cheap storage cost, and many choices of varieties (Pangaribuan, 1994). As potato tuber comes to maturity, starch-rich perimedullary region forms the major portion of the tuber (Gupta and Kaur, 2000), and potato granules are synthesized and stored as roughly spherical shapes in amyloplasts (Naeem *et al*., 1997; Fajardo *et al*., 2013) As the tuber matures, new starch granules are also synthesized in newly produced amyloplasts. There is usually a large granule size distribution within individual tubers in terms of percentages of small, medium, and large granules (Singh *et al.,* 2016). The size of small and large granules ranged from 0.6 to 6 µm and from 10 to 100 µm in potato starch, respectively (Wang *et al.,* 2018), consisting of smooth-surfaced, oval and irregular shape (Singh *et al.,* 2003). Singh *et al.* (2008) reported small starch granules of 1 to 10 µm, medium granules of 11 to 30 µm and larger granules of >30 µm in diameter in four different New Zealand potato cultivars.

Cold storage of potato tuber results in disintegration and disappearance of the amyloplast membranes around the starch granules, which bring in contact with the degradative enzymes such as α-and β- amylases and their substrates (Badenhuizen, 1965; O'donoghue *et al.,* 1995). Thus, prolonged storage of potato tuber at low temperatures can result in starch degradation and conversion of starch into reducing sugar (Zhang *et al.,* 2014). When potato tubers (*Solanum tuberosum*) are stored at temperatures below 9-10°C, the accumulation of sucrose and reducing sugars glucose and fructose occurred because of 'low-temperature sweetening' (LTS) (Pinhero *et al*., 2007). The rate of starch degradation and sugar accumulation depends largely on cultivar and storage temperatures (Kazami *et al*., 2000). Starch content was found to be decreased about 2 times after storage at 0-2°C for 8 weeks in both seed potato and TPS potato tuber by (Karim *et al.,* 2008). A decrease of starch content was also reported after 60-105 days of storage at 4°C in several Indian potato varieties (Yamdeu *et al*., 2015). On the other hand, Biemelt *et al*. (2000) reported that the starch content did not alter throughout the storage period. The starch degradative enzyme not only affects the starch content in cold storage also starch granule. The enzymatic susceptibility of starch granules has been studied by various authors (Franco *et al*., 1988; Srichuwong *et al*., 2005; Adejumo *et al*., 2013). Differences in the enzymatic attack or susceptibilities of starches depend on many factors such as starch source, granule size, extension of association between starch components, rate of amylose and amylopectin, crystalline structure, particle size, surface porosity, type of enzyme (Hoover and Zhou, 2003; Kong *et al*., 2003; Li *et al*., 2004; Tester *et al*., 2006). A shift to lower granule size distribution has been reported in raw starches of different potato varieties after in-vitro enzymatic hydrolysis (Kimura and Robyt, 1995). Cold Induced Sweetening (CIS) susceptible cultivars can cause smaller starch granules when stored for 4 or 12 weeks at 4°C rather than CIS resistant cultivars, which does not change until 24 weeks (Barichello *et al*., 1990). Based on the previous observations, it was hypothesized that when potato tubers are stored at low temperature conditions, starch granule content and size change in relation to starch degradation. Starch granules may change randomly or according to size because several studies showed that the starch granule size is an important factor for influencing the digestibility of raw starch by amylase (Noda *et al*., 2008). Ezekiel *et al.* (2010) observed that the number of small granules decreased, and the number of large granules increased in potato after cold storage. Granule size and surface area affect the hydrolysis rate of starch by amylase, large granules with higher diameter have smaller surface area than small granules with lower diameter and therefore larger granules digested more slowly (Tester *et al*., 2006; Kasemwong *et al*., 2008; Noda *et al.,* 2008). Because of the susceptibility to hydrolytic enzyme attack, deformation like external corrosion, pits or endo-erosion occurred on the starch granule (Planchot *et al.,* 1995).

Even though starch granules change in relation to starch degradation, it is unclear whether and how these happen in case of 'seed tuber' and TPS tuber at the same storage condition. So, the aim of this experiment was to study whether the starch degradation occurs similarly or differently in seed potato and TPS tuber; and how the starch degradation affects the starch granule deformation morphologically in both tubers.

2. Materials and Methods

Plant material

In October 2015, each of 50 potato tubers of

'BARI TPS-1' (TPS) and 'Lady Rosetta' (seed potato) were obtained from Bangladesh. Volume, size, and weight of all the tubers were measured and were stored at 5°C for 0 to 4 months in a refrigerator at the laboratory of the Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Bangladesh. The 'Lady Rosetta' variety is one of the widely cultivated commercial seed potato in Bangladesh, which is also used as processed potato. On the other hand, 'BARI TPS-1' is one of the well-cultivated 'True Potato Seed' variety, which is cultivated from true seed in first year and from the tuberlets from second year. Both of the tuber was collected and cultivated in the experimental plot of Sher-e-Bangla Agricultural University with integrated crop management. Ten tubers per each variety were taken out monthly from the refrigerator, the skin was peeled off and the tubers were grated using a grater of about 45 mm diameter. Half of the grated sample was then blended and washed with desalted water, and filtered through filter paper. This step was repeated 3 times and then filtrated and dried in sunlight under shaded condition for few days when the environmental temperature was around 34°C. This unheated samples were prepared for starch granule observation using SEM (Scanning Electron Microscope). The other half of the grated sample was added to 80% MeOH (100 mL) and heated at 80°C for 30 minutes. The supernatant was decanted, and the residue was extracted by 80% MeOH (100 mL). This step was repeated for 3 times in total and then washed by pure acetone. Then, it was heated and dried on a hot plate at 50 to 70°C only in the daytime and stored at the ambient temperature at night. It took 3 days to dry completely and was weighed repetitively up to constant dry weight. The completely dry sample was cooled to the ambient temperature, stored in a freezer at -20°C. This 80% MeOH (100 mL) extracted samples were used for sugar and starch analysis. The chemical analysis was done at the laboratory of the Department of Bioproduction, Faculty of Agriculture, Yamagata University, Japan.

Soluble sugar content

A 0.2 g of dry powder from each cultivar was taken into a test tube, then 9 mL of 80% MeOH was added, and it was heated at 80°C for 30 min. The extract was centrifuged at 3000 rpm using a centrifuge (KS-500, KUBOTA, and Tokyo) for 10 min, and the supernatant was decanted. This extraction procedure was repeated for 3 times, and the combined supernatant was made up to 50 mL volume with 80% MeOH. Reducing sugar content in 0.5 mL of the solution was measured by Somogyi Nelson method (Nelson, 1944), and the 0.5 mL of the solution was added by 2 units of invertase (pH 4.5, Kanto, Kagaku, Tokyo) and hydrolyzed at 50°C for 30 min. Sucrose content in the solution was also measured using the same technique as reducing sugar. Copper reagent and nelson reagent were used to prepare a standard solution of glucose. Absorbance was measured at 660 nm, and a standard curve was prepared to calculate reducing sugar. Non-reducing sugar was measured from hydrolytic degradation of sucrose, and absorbance was measured at 660 nm.

Abs obtained from the analysis of reducing sugar was denoted by ABS 1 … (1)

Abs obtained from the analysis of non-reducing sugar was denoted by ABS 2 … (2)

Concentration of non-reducing sugar was calculated from = {Abs (2) - Abs (1)} × 0.95 … (3)

Concentration of total soluble sugar was calculated from = (1) + (3)

These steps were repeated for 5 times for each cultivars and for each storage sample.

Starch content

The insoluble solid from the 80% MeOH extract was added with 1.5 mL of distilled water and heated at 100°C for 1 hr. Starch in the pellet was hydrolyzed using amyloglucosidase (Yakult) at 55°C for 3 hrs then neutralized by 0.1% NaOH solution. Starch content was also measured in 0.5 ml of the solution using the same technique of sugar analysis and Glucose Oxidase method. Glucose standard curve was prepared to measure starch content. Absorbance was measured at 660 nm for Somogyi Nelson Method and at 500 nm for Glucose Oxidase method and was repeated for 5 times for each cultivars and for each storage sample.

Abs obtained from the analysis of starch was denoted by Abs … (4)

Starch content was calculated from = Abs 4 × 0.9.

Histological analysis of starch granule

Starch powders were scattered on an adhesive carbon tape and were fixed with 2% osmium tetroxide (OsO_4) and successively washed with 50 mM cacodylate buffer and ultrapure water. The dried samples were coated by Pt using each sample slides by Pt ion coater (JFC-1200, JEOL, Tokyo), and then observed under SEM Scanning Electron Microscope (SEM, TM3000, Hitachi, Tokyo). The length and width

of the starch granules were measured by using Motic Image Plus 2.0 software from SEM images. The length and width ratio of starch granule was measured as the ratio of length:width.

Starch granule was regarded as an ellipsoid, and the volume of starch granule was measured of each axis of the ellipse. For histogram analysis (volume and length/width ratio) 10 SEM image of x 500 magnification and 200 µm across from each treatment were chosen and 5 granules from each image were measured randomly (total 500 starch granules).

Amylose percentage determination

Amylose content was measured using an assay kit (Megazyme, Amylose/Amylopectin Assay Kit, Ireland) according to the procedure outlined by the manufacturer. Percentage of amylose was directly calculated following the specific Megazyme equation based on the measured absorbance values, no additional standard curve or equation was generated for this study. Amylopectin content was calculated by 100% difference of the amylose content (Aristizábal *et al.,* 2007). This step was repeated for 5 times for each cultivar and for each consecutive storage sample.

Statistical analysis

Data were subjected to analysis of variance and the difference between cultivars was compared with t-test using SPSS software.

3. Results

Soluble sugar content

In 'BARI TPS-1' tubers, reducing sugar content reached the highest value (57.80 mg/g DW) after 1 month of storage then decreased rapidly thereafter. Reducing sugar of 'Lady Rosetta' showed the same tendency but the highest value after 1 month of storage (25.86 mg/g dry weight) was less than half of that of 'BARI TPS-1' and decreased gradually thereafter (Fig. 1). Therefore, reducing sugar content of 'BARI TPS-1' was significantly higher than that of 'Lady Rosetta' during 1 to 3 months of storage. Although, sucrose content also showed the highest value after 1 month of storage, the value decreased rapidly thereafter. The decline of 'BARI TPS-1' was slower than that of 'Lady Rosetta', resulting in a significant difference between the two varieties during 2 to 3 months of storage (Fig. 1). Changes in total sugar content were similar to that of sucrose content and significantly higher content was observed also in 'BARI TPS-1' during 2 to 3 months of storage (Fig. 1).

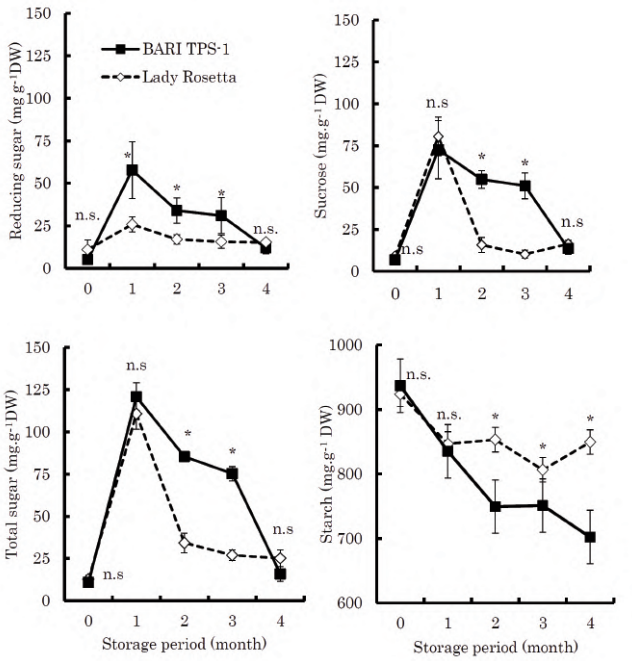

Fig. 1 - Changes in soluble sugar and starch contents of two potato cultivars as affected by different storage period (n = 5 ± SE). * shows significant difference and n.s. shows non-significant different at P<0.05 by student's t-test.

Starch content

Although starch content of both potato varieties decreased continuously throughout the storage period, starch content of 'BARI TPS-1' decreased more rapidly than that of 'Lady Rosetta' variety, resulting in a significant difference from 2 to 4 months of storage (Fig. 1). It is noticeable from figure 1 that decreasing level of starch content was on par with the increasing reducing sugar content, where 'BARI TPS-1' showed rapid starch degradation with higher reducing sugar content after 2 to 4 months of storage than that of 'Lady Rosetta'. Similarly increase in sucrose level of 'BARI TPS-1' after 1 month of storage paralleled with the decreased level of starch content, resulting significant differences between two varieties.

Starch granule sizes

Starch granule length, width, length:width ratio and volume did not change apparently throughout the storage period. Though 'Lady Rosetta' had a tendency to have slightly higher values in length, width and volume, there was no significant difference between the both 'BARI TPS-1' and 'Lady Rosetta' (Fig. 2).

Histogram was shown for starch granule volume

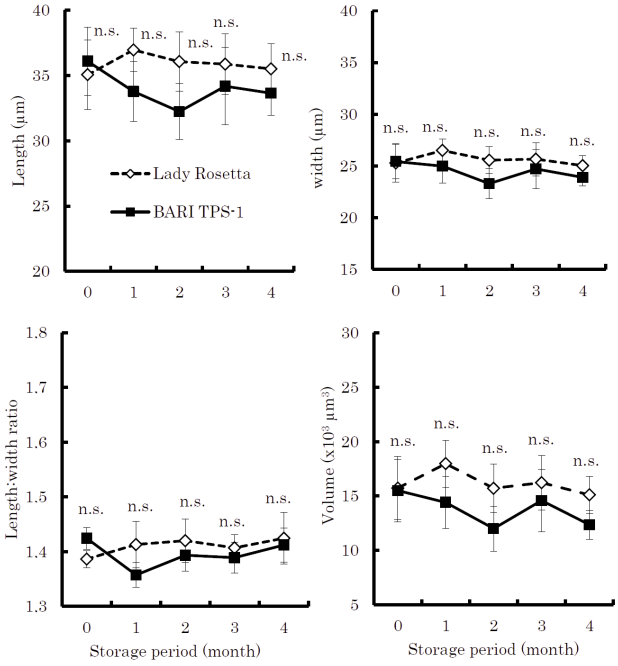

Fig. 2 - Changes in the sizes of starch granule derived from two potato cultivars as affected by different storage period for (n =10±SE). *shows significant difference and n.s. shows non-significant difference at P<0.05 by student's t-test.

and ovality (length:width) within the range of (0.5 to 81.5) x 10^3 µm^3 and (1.05 to 2.22) respectively (Fig. 3 and 4). The highest frequency was observed at (7.6 - 13.5) x 10^3 µm^3 and (1.19 and 1.31) for volume and ovality, respectively and there was no considerable differences between the two cultivars. Moreover, no apparent change also occurred during storage period.

Starch granule morphology

Both 'BARI TPS 1' and 'Lady Rosetta' tubers showed normal and smooth starch granule surface at 0-month storage (Fig. 5). Starch granules of both tubers changed after storage condition. Pit and hole like structures were observed after 1 month storage in both varieties. After 2nd, 3rd and 4th month storages prominent depression or cavity was observed in both potato cultivars. However, both potato tubers showed a similar pattern of deformation.

Amylopectin percentage

Although the percentage of amylopectin in both potato varieties decreased continuously throughout the storage period, Amylopectin of 'BARI TPS-1' decreased more rapidly than that of 'Lady Rosetta' variety, resulting in a significant difference from 2 and 4 months of storage (Fig. 6). Although 'Lady Rosetta' variety had slightly higher percentage than TPS after 3 months of storage, there was no significant difference between them.

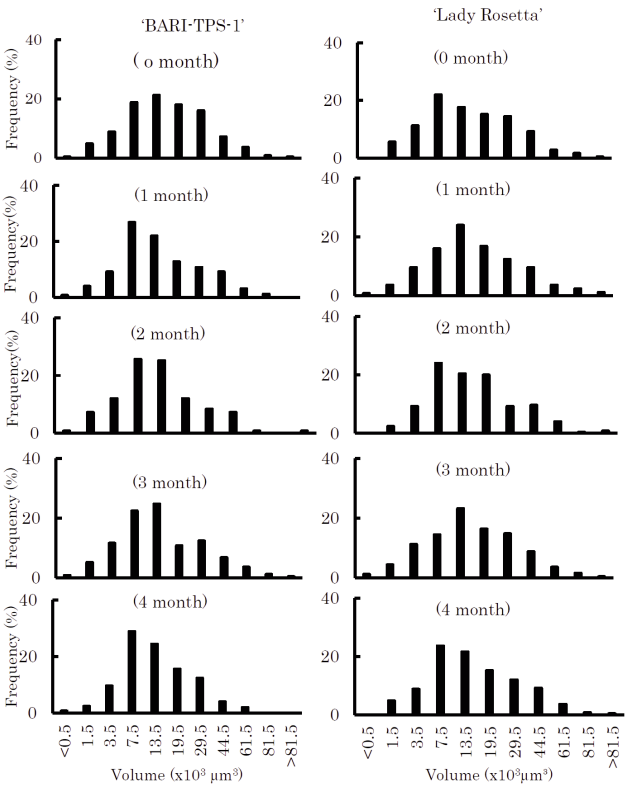

Fig. 3 - Change in histogram for frequency percentage of potato starch granule volume derived from 'BARI-TPS-1' and 'Lady Rosetta' potato varieties as influenced by storage period.

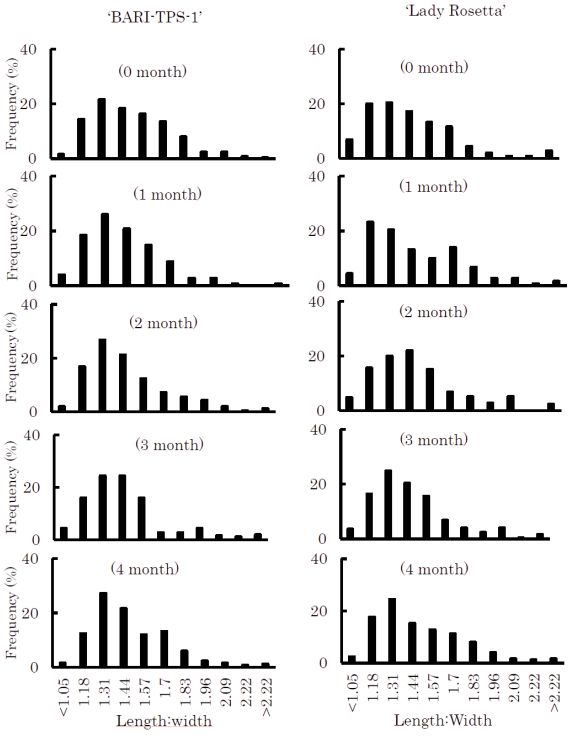

Fig. 4 - Change in histogram for frequency percentage of potato starch granule Length:Width ratio derived from 'BARI-TPS-1' and 'Lady Rosetta' potato varieties as influenced by storage period.

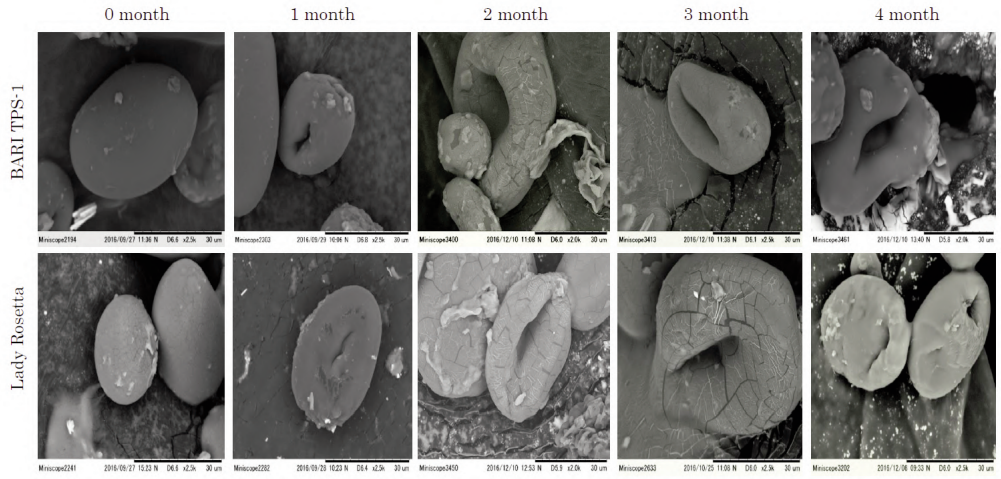

Fig. 5 - Scanning electron micrographs showing the surface morphology of starch granules of 'BARI TPS-1' and 'Lady Rosetta' and potato tuber stored at 5°C at 0 to 4 months of storage.

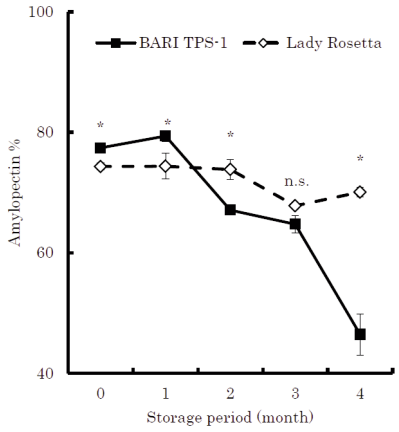

Fig. 6 - Changes in the percentage of amylopectin in 'BARI-TPS-1' and 'Lady Rosetta' potato tubers as influenced by storage period.

4. Discussion and Conclusions

In potato tuber, starch is converted to sugar during cold storage (Malone *et al*., 2006). The starch content of potato tuber decreased markedly during prolonged storage at 4-8°C through the process of conversion of starch into sugars (Nourian *et al*., 2003; Smith *et al*., 2005). Ohad *et al.* (1971) also reported reduction of starch content by 26% after 17 days of storage. Mature dormant potato tuber produced sugars by degradation of a small fraction of starch (Isherwood, 1973). Thus starch content decreased because of the hydrolysis of starch by starch degrading enzymes (Nielsen *et al.*, 1997), suggesting why reducing sugar content increased as starch content decreased during cold storage (Fig. 1). Storage at 0-5°C increases sugar accumulation in potato tuber (Wismer *et al*., 1995; Blenkinsop *et al.,* 2003), which coincides with these experiments where both varieties showed higher reducing sugar content after storage with a significant difference between 'BARI TPS-1' and Lady Rosetta (Fig. 1). This increased level of reducing sugar and decreased level of starch content is related with the increased activity of hydrolysing enzyme (Sowokinos, 2001), which might explain the higher starch degradation in 'BARI TPS-1' (Fig. 1).

During low-temperature storage, the enhancement of sucrose and hexoses (glucose and fructose) levels is known as "cold sweetening' or 'low temperature sweetening', which is an important metabolic process in the roots of many species as well as potato tuber (Wismer *et al*., 1995; Espen *et al*., 1999; Galindo *et al.,* 2004; Galindo *et al.,* 2007). The soluble sugar content of potato tuber increases at low temperature such as 4°C, when stored for 6 to 12 weeks because of starch decomposition and then inclines to decrease (Cochrane *et al*., 1991; Chen *et al*., 2012). Total sugar and sucrose content both showed a similar changing pattern after storage with significantly higher content in 'BARI TPS-1' during 2 and 3 months (Fig. 1). Cold storage condition triggers the tuber starch to breakdown into sucrose through various hydrolytic enzymes which further hydrolyzed into reducing sugars (glucose and fructose) (Sowokinos, 2001). The amount of soluble sugar accumulated in

tuber also depends on different cultivars (Zommick *et al*., 2014).), which could explain the higher reducing sugar in 'BARI TPS-1' after storage than Lady Rosetta (Santos *et al*., 2020).

However, there was a sudden increase in starch content between 3rd to 4th months of storage in 'Lady Rosetta' variety (Fig. 1), suggesting recondition of starch or increase of respiration losses during storage. The difference in respiration rates depend on cultivar, growing conditions, experimental conditions, and physiological status of the tubers (Fennir *et al*., 2003). Comparatively lower starch degradation after 3 to 4 months of storage might have contributed to lower presence of reducing sugar in 'Lady Rosetta' (Fig. 1). This can also be explained that likely the rise of starch content between 3 and 4 months of storage, amylopectin percentage also showed similar kind of tendency in 'Lady Rosetta' variety (Fig. 6). An increase in amylopectin percentage from 3 to 4 months of storage suggested lower enzymatic degradation (Fig. 6) (Hofvander *et al.,* 2004). It seemed amaylase enzyme activity reduced during 3 to 4 months of storage because of sprouting of seed potato varieties or it can be explained that the amaylase enzyme activity was not enough to degrade the starch in seed potato (Lewis *et al.,* 1994). As the experimental condition was same for both of the tubers, hence these may explain faster physiological aging in seed potato variety than TPS variety. This may also suggest that experimental storage temperature might have affected the respiration losses; and the sugar had been used for tuber germination in case of seed tuber (Olsen *et al.,* 2003).

The TPS variety showed higher starch degradation rate than the seed potato (Fig. 1), and sharp decrease in amylopectin percentage after storage (Fig. 6), moreover there was higher percentage of amylopectin degradation in 'BARI TPS-1' than 'Lady Rosetta' starting after 1 month of storage and continued as the storage progressed (Fig. 6), but there was no apparent changes in starch granule volume and ovality (legth:width ratio) (Fig. 2). This result suggested a non-uniform degradation pattern of starch granules in ''BARI TPS-1'and 'Lady Rosetta' varieties.

Histograms were also carried out to investigate, if the starch granule deformation primarily started in smaller sized granules, or larger sized granules (Figs. 3 and 4). Singh *et al.* (2008) reported a shift of granule size range to smaller granule sizes in isolated starches of New Zealand Taewa (Maori potato) when they were stored for three and six months at 4°C temperature. This experiment also reported erosion and pitting on the surface of stored potato starch. In case of banana, surface erosion by enzymatic degradation resulted in smaller granules with elongated shape and exo-corrosion process caused pits on the surface of the starch granule with high frequency (Peroni-Okita *et al.,* 2013). This shift of granule size distribution has been related to rapid digestion of starch granule and composition variation between small and large granules (Salunkhe *et al.,* 1989). However, there were no considerable changes of starch granule sizes in both potato cultivars in our study. This suggested that, in spite of having significant change in starch degradation along storage in both variety, it had hardly influenced the size of starch granule (Fig. 2, 3 and 4).

Moreover, it was found that even after 4 months of storage periods, there was no significant change in starch granule sizes in both cultivars, in spite of 'Lady Rosetta' having slightly higher tendency in the value of length, width and volume (Fig. 2). Fajardo *et al.* (2013) also reported unchanged granule size after storage, which was attributed to the nearly undetectable change in volume of starch granule. Russet Burbank potato tubers also showed similar granular size distribution after storage at 3.9°C (Johnston *et al.,* 1968).

SEM observation showed that starch granules from both harvested tubers presented smooth granular surface under SEM without any storage treatment or at 0-month storage (Fig. 5). Although there is argument about the appearance of natural potato starch granular surface, several studies showed smooth granular surface of natural potato starch under microscopic observation. Cottrell *et al.* (1993) reported smooth granular surface of potato starch of Record and Brodick potato cultivar at harvest. (Sarikaya *et al*., 2000) also reported smooth granular surface of potato starch before any enzymatic or freezing treatment. As the SEM observation in this experiment showed hole or pit formation on granular surface of both varieties after storage (Fig. 5) (Sarikaya *et al*., 2000; Noda *et al*., 2005), it is suggested that the long term storage at low temperature of this experiment lowered the starch content which led to susceptibility to hydrolytic enzyme attack that changes the properties and composition of starch granule (Barichello *et al.* 1990). The cold storage temperature might have given damage to amyloplast membrane by starch hydrolysis (Ohad *et al.,* 1971), which enhanced membrane permeability through

starch hydrolysis enzymes, and resulted the formation of cracked region or surface hole on the granule (Sujka and Jamroz, 2010).

However, no apparent change was observed in the ratio of granules and histogram analysis of both potato starch granule, but the SEM observation clearly showed deformation of starch granule forming hole or pit like structures gradually started from 1 month of storage and continued to 4 months of storage (Fig. 5). On the other hand, gradual starch degradation and decreased amylopectin percentage indicated activation of enzymatic degradation in both cultivars after storage (Fig. 1 and 6). Therefore, it is suggested that starch granule erosion may occur at specific surface region of the granule causing no change in the granular size. Starch hydrolyging enzyme like a-amylase might have attacked at particular points on the granule surface, forming tunnels into the granule, thus hydrolyzed the granule from the inside (Lindeboom *et. al*., 2004). Similar finding was reported by Peroni-Okita *et al.* (2013) where starch granules of green banana maintained the rounded shape after low temperature but presented pits on the granule surface which was enlarged by the corrosion process, suggesting partial degradation of starch granules.

Thus enzyme molecules can affect the starch granule in different patterns either by forming pin holes, medium sized hole, sponge like erosion or selected point at the surface leading to a single hole (Sujka and Jamroz, 2010). Enzyme can gain access to innermost region of the starch granule by faster digestion than at the periphery region of the granule, which can form shallow hole like structure (Duffus, 1984). This may explain how the both starch granules showed no changes in their sizes despite of granular deformation.

However, 'BARI TPS-1' showed a higher starch degradation percentage throughout the storage period than the seed tuber potato. This suggested higher susceptibility of starch degrading enzyme in TPS tuber than that of seed potato tuber. Variation in starch granule morphology and their crystalline organization may explain the susceptibility to enzymatic degradation (Gallant *et al*., 1992). Amylase plays the major role in in-vivo breakdown of starch (Manners, 1985). Amylose and amylopectin ratio may explain the degradation pattern of starch granules in both TPS and seed potato varieties. Both the potato tuber showed decreased percentage of amylopectin along the storage period, whereas 'BARI TPS-1' resulted lower amylopectin percentage than the seed potato (Fig. 6). Bach *et al*. (2013) described that as amylopectin degrades more rapidly than amylose, it causes activation of more starch degrading enzymes. Therefore, 'BARI TPS-1' might have allowed deeper penetration of starch degrading enzymes than the seed tuber in spite of no apparent changes in granule size (Fig. 5). As the extent of pit or cavity of starch granules were not determined in this experiment, it can be suggested that probably a different inner molecular organization of TPS starch granule may be allowed differentiated enzyme attack, which could be responsible for slightly smaller starch granule than 'Lady Rosetta' during Storage (Fig. 6) with no significant difference. This also suggests that starches vary in their resistance to enzymatic susceptibility (Srichuwong *et al*., 2005).

There are different opinions about the presence of pores or holes on the surface of potato starch granules. Although several observations concluded that some factors could cause an increase in the number and size of pores on starch granules surface (Fannon *et al*., 1992). Moreover, the expansion of granule degradation increased with the increased enzyme concentration (Mu *et al*., 2015). During enzymatic hydrolysis, some regions of granules are more susceptible to enzyme attack because of less organized amorphous rings, whereas the crystalline lamella provides higher resistance to enzymatic erosion (Oates, 1997). This kind of enzymatic hydrolysis was characterized by forming a hole by creating channel through the less resistant granule core (Jung *et al*., 2017), which may explain deeper enzyme penetration in TPS tuber. Action pattern of both endo- and exo-amylase at a lower temperature may explain overall amylolytic activity in TPS and seed potato tubers (Shin *et al*., 2002; Nabubuya *et al.,* 2012).

4. Conclusions

In this experiment, the starch content of TPS tuber degraded more rapidly and produced higher reducing sugar content than the seed potato variety after storage. This result indicated that, TPS tuber may not be acceptable for low temperature storage compare to seed potato variety. There were no significant changes in granule size and volume, indicated that granule did not change as a ratio in both tubers. However, both potato granules deformed by forming holes or cavities at innermost surface region

after storage, suggesting the starch granule degraded partially rather than concentrically. As both the tubers showed a similar kind of degradation pattern of starch granule in spite of higher starch degradation rate in TPS tuber, similarly the rapid degradation rate of amylopectin in TPS tuber explained higher amylase activities in TPS than the seed tuber. This result suggested possibilities of deeper penetration in TPS starch granule than the seed tuber starch granules. Further studies on the susceptibility of starch degrading enzyme on TPS and seed potato can be done to foresee the starch degradation pattern in TPS and seed potato tuber.

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References

ADEJUMO A.L., ADERIBIGBE, OWOLABI R.U., 2013 - *Comparitive studies of starch susceptibilities to α-amylase degradation of different cereal and root crops of Nigeria.* - Afr. J. Biotechnol., 12(29): 4663-4669.

ARISTIZABAL J., SANCHEZ T., LORIO D.M., 2007 - *Guía técnica para producción y análisis de almidón de yuca: Organización de las Naciones Unidas para la Agricultura y la Alimentación Roma.* - ISBN: 92-5-305677-0

BACH S., YADA R.Y., BIZIMUNGU B., FAN M., SULLIVAN J.A., 2013 - *Genotype by environment Interaction effects on starch content and digestibility in potato (*Solanum tuberosum L.*).* - J. Agric. Food Chem., 61(16): 3941-3948.

BADENHUIZEN N.P., 1965 - *Occurrence and development of starch in plants*. - In: WHISTLER R.L., and E.F. PASCHALL (eds.) *Starch: Chemistry and technology.* Academic Press, New York, pp. 65-104.

BARICHELLO V., YADA R.Y., COFFIN R.H., STANY D.W., 1990 - *Low temperature sweetening in susceptible and resistant potatoes: Starch structure and composition.* - J. Food Sci., 55(4): 1054-1059.

BIEMELT S., HAJIREZAEI M., HENTSCHEL E., SONNEWALD U., 2000 - *Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties*. - Potato Res., 43(4): 371-382.

BLENKINSOP R.W., COPP L.J., YADA R.Y., MARAGONIA G., 2003 - *A proposed role for the anaerobic pathway during low-temperature sweetening in tubers of* Solanum tuberosum*.* - Physiol. Plant., 118(2): 206-212.

CHEN X., SONG B., LIU J., YAN J., H.E. T., LIN Y., ZHANG, H., XIE C., 2012 - *Modulation of gene expression in cold-induced sweetening resistant potao species* Solanum berthaultii *exposed to low temperature*. - Mol. Genet. Genomics., 287: 411-421.

COCHRANE M.P., DUFFUS C.M., ALLISON M.J., MACKAY G.R., 1991 - *Amylolytic activity in stored potato tubers. 1. Estimation usingp-nitrophenyloligosaccharides*. - Potato Res., 34: 325-332.

COTTRELL J.E., DUFFUS C.M., MACKAY G.R., ALLISON M.J., 1993 - *Changes in the surface morphology of starch granules of the cultivated potato,* Solanum tuberosum *L. during storage*. - Potato Res., 36: 119-125.

DUFFUS C.M., 1984 - *Metabolism of reserve starch*. - In: LEWIS D.H. (ed.) *Storage carbohydrates in vascular plants. Society for Experimental Biology Seminar Series*, pp. 231-252.

ESPEN L., MORGUTTI S., ABRUZZESE A., NEGRINI N., RIVETTA A., QUATTRINI M.M., COCUCCI M., COCUCCI S. M., 1999 - *Changes in the potato (*Solanum tuberosum *L.) tuber at the onset of dormancy and during storage at 23°C and 3°C. I. Biochemical and physiological parameters.* - Potato Res., 42(2): 189-201

EZEKIEL R., RANA G., SINGH N., SINGH S., 2010 - *Physico-chemical and pasting properties of starch from stored potato tubers.* - J. Food Sci. Technol., 47(2): 195-201.

FAJARDO D., HAYNES K.G., JANSKY S., 2013 - *Starch characteristics of modern and heirloom potato cultivars.* - Am. J. Potato Res., 90: 460-469.

FANNON J.E., HAUBER R.J., BEMILLER J.N., 1992 - *Surface pores of starch granules.* - Cereal Chem., 69: 284-288.

FENNIR M.A., LANDRY J.A. RAGHAVAN G.S.V., 2003 - *Development and testing of an automated infrared gas analysis system for postharvest respiration study*. - Appl. Eng. Agric., 19(3): 335-340.

FRANCO C.M.L., CIACCO C.F., TAVARES D.Q., 1988 - *Studies on the susceptibility of granular Cassava and corn starches to enzymatic attack. Part 2. Study of the granular structure.* - Starch., 40(1):29-32.

GALINDO F.G., HERPPICH W., GEKAS V., SIOHOLM I., 2004 - *Factors affecting quality and postharvest properties of vegetables: integration of water relations and metabolism*. - Crit. Rev. Food Sci. Nutr., 44(3): 139-154.

GALINDO F.G., SIOHOLM I., RASMUSSON A.G., WIDELL S., KAACK K., 2007 - *Plant stress physiology: opportunities and challenges for the food industry*. - Crit. Rev. Food Sci. Nutr., 47(8): 749-763.

GALLANT D.J., BOUCHET B., BULEON A., PEREZ S., 1992 - *Physical characteristics of starch granules and susceptibility to enzymatic degradation*. - Eur. J. Clin. Nutr., 46(2): 3-16.

GUPTA A.K., KAUR N., 2000 - *Carbohydrate reserves in plants: synthesis and regulation*. - Elsevier, Ludhiana,

India, pp. 223-248.
HOFVANDER P., ANDERSSON M., LARSSON C.T., LARSSON H., 2004 - *Field performance and starch characteristics of high-amylose potatoes obtain, ed by antisense gene targeting of two branching enzymes.* - Plant Biotech. J., 2(4): 311-320.
HOOVER R., ZHOU Y., 2003 - In vitro *and* in vivo *hydrolysis of starches by α-amylase and resistant starch formatin in legumes* - a review. - Carbohydr. Polym., 54(4): 401-417.
ISHERWOOD F.A., 1973 - *Starch-sugar interconversion in* Solanum tuberosum*.* - Phytochem., 12(11): 2579-2591.
JOHNSTON F., URBAS B. KHANZADA G., 1968 - *Effect of storage on the size distribution and amylose/amy, lopectin ratio in potato starch granules*. - Amer. Potato J., 45(9): 315-321.
JUNG Y.S., LEE B.H., YOO S.H., 2017 - *Physical structure and absorption properties of tailor-made porous starch granules produced by selected amylolytic enzymes*. - PloS one, 12(7): 1-14.
KARIM M.R., KHAN M.M.H., UDDIN M.S., SANA N.K., NIKKON F., RAHMAN M.H., 2008 - *Studies on the sugar accumulation and carbohydrate splitting enzyme levels in post harvested and cold stored potatoes*. - J. Biol. Sci., 16: 95-99.
KASEMWONG K., PIYACHOMKWAN K., WANSUKSRI R. SRIROTH K., 2008 - *Granule sizes of canna (*Canna edulis*) starches and their reactivity toward hydration enzyme hydrolysis and chemical substitution*. - Starch - Stärke, 60(11): 624-633.
KAZAMI D., TSUCHIYA T., KOBAYASHI Y., OGURA N., 2000 - *Effect of storage temperature on quality of potato tubers*. - J. Japanese Soc. Food Sci. Technol., 47(11): 851-856.
KIMURA A., ROBYT J.F., 1995 - *Reaction of enzymes with starch granules: kinetics and products of the reaction with glucoamylase.* - Carbohydr. Res., 277(1): 87-107.
KONG B.W., KIM J.I., KIM M.J., KIM J.C., 2003 - *Porcine pancreatic alpha-amylase hydrolysis of native starch granules as a function of granule surface area*. - Biotechnol. Prog., 19: 1162-1166.
LEWIS C.E., LANCASTER J.E., MEREDITH P., WALKER J.R.L., 1994 - *Starch metabolism during growth and storage of tubers of two New Zealand potato cultivars.* - J. Crop Hort. Sci., 22(3): 295-304.
LI J.H., VASANTHAN T., HOOVER R., ROSSNAGEL B.G., 2004 - *Starch from hull-less barley: V.* in-vitro *susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase.* - Food Chem., 84(4): 621-632.
LINDEBOOM N., CHANG P.R., TYLER R.T., 2004 - *Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: A review.* - Starch, 56(34): 89-99.
MALONE J.G., MITTOVA V., RATCLIFFE R.G. KRUGER N.J., 2006 -*The response of carbohydrate metabolism in potato tubers to low temperature*. - Plant Cell Physiol., 47(9): 1309-1322.
MANNERS D.J., 1985 - *Starch,* pp. 149-204. - In: DEY P.M., and R.A. DIXON (eds.) *Biochemistry of storage carbohydrates in green plants.* Academic Press, London, UK, pp. 378.
MU T.-H., ZHANG M., RAAD L., SUN H.-N., WANG C., 2015 - *Effect of α-amylase degradation on physicochemical properties of pre-high hydrostatic pressure-treated potato starch.* - PLOS ONE., 10: e0143620.
NABUBUYA A., NAMUTEBI A., BYARUHANGA Y., NARYAHUS J., STENSTROM Y., WICKLUND T., 2012 - *Amylolytic activity in selected sweetpotato (*Ipomoea batatas *Lam) varieties during development and in storage*. - Food Nutr. Sci., 3(5): 660-668.
NAEEM M., TETLOW I.J., EMES M.J., 1997 - *Starch synthesis in amyloplasts purified from developing potato tubers.* - Plant J., 11(5): 1095-1103.
NELSON N.A., 1944 - *Photometric adaptation of the Somogyi method for the determination of glucose.* - J. Biol. Chem., 153: 375-380.
NIELSEN T.H., DEITING U., STITT M., 1997 - *A [beta]-amylase in potato tubers is induced by storage at low temperature.* - Plant Physiol., 113(2): 503-510.
NODA T., TAKIGAWA S., MATSUURA-ENDO C., KIM S.J., HASHIMOTO N. YAMAGUCHI H., HANASHIRO I., TAKEDA Y., 2005 - *Physicochemical properties and amylopectin structures of large, small, and extremely small potato starch granules.* - Carbohydr. Polym., 60(2): 245-251.
NODA T., TAKIGAWA S., MATSUURA-ENDO C., SUZUKI T., HASHIMOTO N., KOTTEARACHCHI N.S., ZAHIDUL I.S.M., 2008 - *Factors affecting the digestibility of raw and gelatinized potato starches.* - Food Chem., 110(2): 465-470.
NOURIAN F., RAMASWAMY H.S., KUSHALAPPA A.C., 2003 - *Kinetics of quality change associated with potatoes stored at different temperatures.* - LWT - Food Sci. Technol., 36(1): 49-65.
OATES C.G., 1997 - *Towards an understanding of starch granule structure and hydrolysis.* - Trends Food Sci. Technol., 8: 375-382.
O'DONOGHUE E.P., YADA R.Y., MARAGONI A.G., 1995 - *Low temperature sweetening in potato tubers: the role of the amyloplast membrane.* - J. Plant Physiol., 145: 335-341.
OHAD I., FRIEDBERG I., NE'EMAN Z. SCHRAMM M., 1971 - *Biogenesis and degradation of starch: I. The fate of the amyloplast membranes during maturation and storage of potato tubers.* - Plant Physiol., 47(4): 465-477.
OLSEN N., THORNTON R.E. BARITELLE A., HYDE G., 2003 - *The influence of storage conditions on physical and physiological characteristics of Shepody potatoesa*. - Potato Res., 46(1): 95-103.
PANGARIBUAN D.H., 1994 - *The effects of planting depth and hilling upon the growth and yield of potatoes*

grown from true potato seed (TPS). - Acta Horticulturae, 369: 358-367.

PERONI-OKITA F.H.G., CARDOSO M.B., AGOPIAN R.G.D., LOURO R.P., NASCIMENTO J.R.O. PURGATTO E., TAVARES M.I.B., LAJOLO F.M., CORDENUSI B.R., 2013 - *The cold storage of green bananas affects the starch degradation during ripening at higher temperature*. - Carbohydr. Polym. 96(1): 137-147.

PINHERO R.G., COPP L.J., AMAYA C.L., MARAGONI A.G., YADA R.Y., 2007 - *Roles of alcohol dehydrogenase, lactatedehydrogenase and pyruvate decarboxylase in low-temperature sweetening in tolerant and susceptible varieties of potato* (Solanum tuberosum). - Physiol. Plant., 130(2): 230-239.

PLANCHOT V., COLONNA P., GALLANT D.J., BOUCHET B., 1995 - *Extensive degradation of native starch granules by alpha-amylase from* Aspergillus fumigatus. - J. Cereal Sci., 21: 163-171.

SALUNKHE D.K., DESAI B.B., CHAYAN J.K., 1989 - *Potatoes*. - In: ESKIN N.A.M. (ed.) *Quality and preservation of vegetables.* CRC Press Inc, Boca Raton, Florida, pp. 1-52.

SANTOS M.N.S., ARAJO F.F., LIMA P.C.C., COSTA L.C., FINGER F.L., 2020 - *Changes in potato tuber sugar metabolism in response to natural sprout suppressive compounds*. - Acta Sci. Agron., 42: 1-6.

SARIKAYA E., HIGASA T., ADACHI M., MIKAMI B., 2000 - *Comparison of degradation abilities of α- and ß-amylases on raw starch granules.* - Process Biochem., 35(7): 711-715.

SHIN K.S., CHAKRABARTY D., PAEK K.Y., 2002 - *Sproutingrate, change of carbohydrate contents and related enzymesduring cold treatment of lily bulblets regenerated* in vitro. - Sci. Hortic., 96: 195-204.

SINGH J., COLUSSI R., McCARTHY O.J. KAUR L., 2016 - *Potato Starch and Its Modification.* - In: SINGH J., and L. KAUR (eds.) *Advances in potato chemistry and technology.* Academic Press, Cambridge, Massachusetts, USA, pp. 195-247.

SINGH J., McCARTHY O.J., SINGH H., MOUGHAN P.J., 2008 - *Low temperature post-harvest storage of New Zealand Taewa (Maori potato): Effects on starch physico-chemical and functional characteristics.* - Food Chem., 106(2): 583-596.

SINGH N., SINGH J., KAUR L., SINGH SODHI N., SINGH GILL B., 2003 - *Morphological, thermal and rheological properties of starches from different botanical sources*. - Food Chem., 81(2): 219-231.

SMITH A.M., ZEEMAN S.C., SMITH S.M., 2005 - *Starch degradation*. - Annu. Rev. Plant Biol., 56: 73-98.

SOWOKINOS J.R., 2001 - *Biochemical and molecular control of cold-induced sweetening in potatoes*. - Am. J. Potato Res., 78(3): 221-236.

SRICHUWONG S., SUNARATI T. C., MISHIMA T., ISONO N., HISAMATSU M., 2005 - *Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties.* - Carbohydr. Polym., 62(1): 25-34.

SUJKA M., JAMROZ J., 2010 - *Characteristics of pores in native and hydrolyzed starch granules.* - Starch., 62(5): 229-235.

TESTER R.F., QI X., KARKALAS J., 2006 - *Hydrolysis of native starches with amylases.* - Anim. Feed Sci. Technol., 130(1-2): 39-54.

WANG J., GUO K., FAN X., FENG G., WEI C., 2018 - *Physicochemical properties of C-type starch from root tuber of* Apios fortunei *in comparison with maize, potato, and pea starches.* - Molecules, 23(9): 2132.

WISMER W.V., MARAGONI A.G., YADA R.Y., 1995 - *Low-temperature sweetening in roots and tubers.* - Hortic. Rev., 17: 203-231.

YAMDEU J.H.G., GUPTA P.H., SHAH A.K., PATEL N.J., TALATI J.G., 2015 - *Effect of storage temperature on carbohydrate metabolism and development of cold-induced sweetening in indian potato (*Solanum Tuberosum *L.) varieties*. - J. Food Biochem., 40(1): 71-83.

ZHANG H., HOU J., Liu XIE C., SONG B., 2014 - *Amylase analysis in potato starch degradation during cold storage and sprouting.* - Potato Res., 57(1): 47-58.

ZOMMICK D.H., KNOWELS L.O., PAVEK M.J., KNOWLES N.R., 2014 - *In-season heat stress compromises postharvest quality and low-temperature sweetening resistance in potato* (Solanum tuberosum *L.)*. - Planta, 239(6): 1243-1263.

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Effect of salt stress by "onsen" water on plant growth and fruit quality of tomato cv. Reika in pot soil

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Abstract: Salt stress often can enhance the fruit quality of tomatoes. Seawater is one of the substrates used by growers. However, utilization of seawater on tomato production is difficult in the hinterland as it is far away from the seaside. Some "onsen" water also show a high salt concentration (2%). Therefore, it could also be used as a substrate of salt stress treatment. In this study, salt stress was provided by Yupoka "onsen" water, and the effects of different nutrient ECs on plant growth and fruit quality of tomatoes were investigated. Tomato plants 'Reika' were grown in pot soil, and nutrients with EC 2, 4, 8 and 12 mS/cm were applied at the time of irrigation. The fruits were harvested at turning stage until the 3rd truss. Soil salinity attained EC 3.6, 6.7, 12.8, and 15.6 mS/cm. SSC, organic acid, dry matter and NO_3^- increased by 50, 79, 50 and 27%, respectively at EC12 mS/cm while, weight, size, and water content decreased up to 40, 20, and 4%, respectively. However, fruit cracking did not occur apparently. Most of the plant growth parameters were reduced.

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated horticultural crops in the world (FAO, 2016). Tomato fruits are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres and thus contribute to a healthy, well-balanced diet. The majority of tomato fruits produced are consumed in processed form such as peeled tomato (whole or diced), juices, sauce and ketchup, whose manufacture often requires peel removal (Shankara *et al*., 2005; Rock *et al*., 2012). Improvement of fruit quality is an urgent issue for tomato growers and consumers (Ho, 1999; Lu *et al*., 2019). Sugars and organic acids are the most important factors for determining the fruit quality (Lu *et al*., 2019). Soluble Solids Content (SSC) of ripe tomato fruit is usually 3 to 5% and can reach values over 10% (Balibrea *et al.,* 2006; Gautier *et al*., 2010). In Japan, "Shio" or salt tomatoes, are cultivated in the area of Uto and Yatsushiro in Kumamoto Prefecture. They are grown on drained land, rich in

salt and other minerals. The wholesale price of "Shio" tomato (have SSC of 10% or higher) is often ten times higher than common tomatoes (http://higoayuminokai.co.jp/sawamura.html).

High electrical conductivity (EC) treatment is a well-known technique to increase SSC of tomato fruit because the decreased osmotic potential of nutrient solution restricts transport of water to fruit (Adams *et al*., 1991; Cuartero and Fernandez-Munaoz, 1999; Shabala *et al*., 2012). However, plant exposed to salt stress for long time suffer from phyto-toxicity due to NaCl accumulation. This leads to nutrition imbalance and the uptake of some ions is inhibited (Zhang *et al*., 2017). Amjad *et al*. (2014) showed a linear decline of the absorption of macro and micro ions by fruit as EC and truss position become higher. The plant growth and fruit yield began to decline when the nutrient solution exceeded EC 2.5-4.0 mS/cm (Bustomi *et al*., 2014). In previous studies, EC 5.5 m/.cm treatment reduced the tomato yield by 22.4~31.1%, 12.6~28.0% and 11.7~27.3% in 2012, 2013 and 2014, respectively (Zhai *et al*., 2015), but SSC increased (Oztekin and Tuzel, 2011; Zhang *et al*., 2017). Other studies reported in soil-less tomato culture that SSC increased while fruit weight decreased at high salinity (Magán *et al*., 2008; Kamrani *et al*., 2013). Although tomato growers commonly use NaCl or seawater to increase EC, it is well known that some "onsen" like Haguro has about 2% of NaCl. Therefore, it may be used as an alternative source of salt stress. This research was conducted to assess if high quality tomato fruit can be grown under salt stress by "onsen" water.

2. Materials and Methods

Four different salt stress treatment (EC 2, 4, 8, and 12 mS/cm) were evaluated on tomato plant 'Reika' during spring to summer in 2016. "Onsen" water obtained from Yupoka in Haguro town, Yamagata/Japan was used as it has a high salty level (EC 40 mS/cm of EC, 2% of NaCl and pH 7.2). Initially, the seeds were sown on moist papers in petri dishes on 5 April, 2016 and maintained in a controlled growth chamber (24°C; 14/10 h light/dark photoperiod; RH 45-50%) to induce germination. On 08 April, the germinated seeds were transferred in cell tray (100 ml) in a glass-house and twenty days after (on 28th April), the seedlings were transplanted into 500 ml pots filled with a planting medium (Baido 300 g containing 0.22 g/l of nitrogen). The seedlings were maintained in the same glass-house (18.7°C; RH 76.8%). On 17 May, twenty tomato seedlings with 5-7 expanded leaves were further transplanted into plastic pots (25 l) containing Baido 300 g and 300 g of organic fertilizer with 3% of nitrogen (Pro-Bokash-KantoNosan, Tochigi) and maintained in a green-house. One month (On 16th June) after transplanting (flowering stage), the plants were treated with different concentration of "onsen" water (EC 2, 4, 8, and 12 mS/cm) replicated five times. The experiment was laid out in Randomized Complete Block Design (RCBD). The EC 2 was 100 g of Hyponex NPK $_{6\text{-}10\text{-}5}$ diluted in 100 l of water. For other treatments (EC 4, 8 and 12), "onsen" water was added into EC 2. The EC meter (LAQUAtwin EC, Horiba,Tokyo) was used to adjust the solution EC. The applied solution was EC 2±0.1, EC 4±0.1, EC 8±0.1, and EC 12±0.1. The nutrient solutions were prepared every week.

Watering was done daily using tap water during the first month (1.8 l/pot). For control of soil/root insects, G F Orthoran (Bifenthrin) insecticide was applied once during the season at rate of 5 g/pot. While for the control of above ground insects, Frutrifol+Tebuconazole, best guard (1 g/l) mixed with a sticker as adjuvant (0.5 ml/l) was also applied every two weeks. Plants were kept as single stem with five trusses and five fruits per truss. Fruits were harvested from three trusses by taking two fruits on each at green maturity until the end of harvest (4 Aug. 2016). The daily average temperature was 24.1°C and RH was 74.2% in the green-house during the growing season.

Plant height, stem size, leaf size, thickness, and SPAD were measured once a week during the plant cultivation from first "onsen" water application until first harvesting. After "onsen" water application, approximately 2 l of tap water were applied once a week into soil pots, and the exudate was collected. EC, pH, NaCl and NO_3^-ion concentrations in the exudate were measured using EC meter (LAQUAtwin EC, Horiba,Tokyo), pH meter (LAQUAtwin pH, Horiba, Tokyo), nitrate ion meter (LAQUAtwin NO_3^-, Horiba, Tokyo) and, NaCl meter (CM-14 P, TOA, Tokyo). For the chemical analysis (SSC, organic acid and NO_3^-ion), harvested fruits with same skin color selected using Color Reader (CR-10, Konica Minolta, Tokyo) were cut into half longitudinally. Thereafter, samples were taken transversally using cork borer, grinded in the mortar and filtrated by tissues filter. The extracts were centrifuged at 6000 rpm for one minute. SSC, organic acid and NO_3^-were measured by Digital

refract-meter (PR-101, Atago, Tokyo), organic acid meter (PAL-BXIACID F5, Atago, Tokyo) and nitrate ion meter (LAQUAtwin NO_3^- B-341, Horiba, Tokyo) respectively.

Before measurement, the respective meters were opened and the glass electrodes calibrated with distilled water/or standard solution followed by rinsing with distilled water and wiping using paper towels. Extracted juice per sample was filled slowly on the glass electrode without bubbling, and the measurements were recorded. Prior to measuring, the juice for organic acid was diluted indeionized water using a ratio of 1:50 (0.1 ml of juice into 4.9 ml of desalted water). SSC was expressed in % Brix, organic acid in %, and NO_3^- in ppm.

Half of the harvested fruit samples were frozen at -20°C for ten days. The samples were dried using Eyela Freeze Dryer FDU-540. The wet and dry samples were weighed in order to determine fruit water and dry matter content. The dried ones had been powdered in mortar using liquid nitrogen. One gram of the powdered sample was put in a crucible, and incinerated at 200°C for 2 hours, 550°C for 10 hours, and 200°C for 2 hours, respectively. Fruit ash content was calculated by weighing the sample.

Data analysis were performed using the Statistical Analysis Software package, GenStat 19th edition at 5% level of significance. Least significant difference test (LSD) was used to separate treatment means.

3. Results

The average temperature was 24°C and RH 74% in the green house during tomato cultivation. The difference in plant height among salt stress treatment was observed after about three weeks "onsen" water application. Fruit SSC, organic acid and NO_3^- increased by about 50, 79 and 26.5%, respectively in EC12 mS/cm while SSC: OA ratio was not affected by high EC12 mS/cm (Fig. 1 and 2). Tomato plants treated with EC2 mS/cm and EC4 mS/cm produced bigger fruit than EC8 mS/cm and EC12 mS/cm (Fig. 1 and 2). The fruit weight, size decline up to 50% whilst dry matter almost doubled in EC12 mS/cm (Fig. 2). The EC, salt and NO_3^- concentration in culture medium increased by 53.9, 70.3, and 52% at higher EC because of high amount of "onsen" water applied. The high pH of "onsen" water (pH 7.2) did not affect the culture medium pH in high EC. The difference in plant height among salt stress treatment was

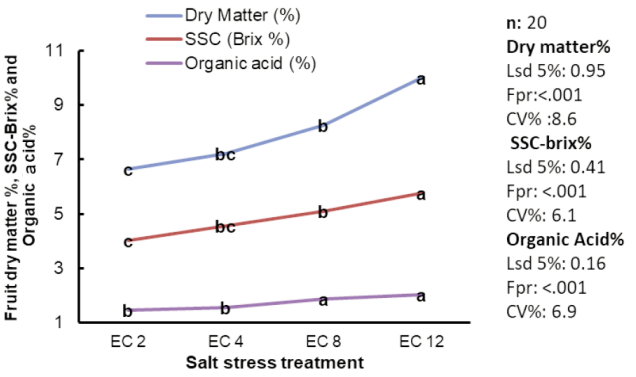

Fig. 1 - Effect of different concentration of "onsen" water on fruit SSC Brix%, dry matter and organic acid of tomato plants variety 'Reika' in the greenhouse.

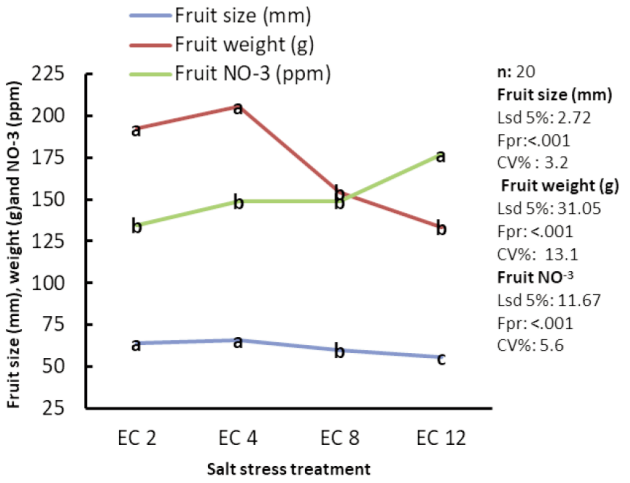

Fig. 2 - Effect of different concentration of "onsen" water on fruit size, weight and NO^{-3} of tomato plants variety 'Reika' in the greenhouse.

observed after about three weeks "onsen" water application. The curve indicates that tomato plants treated with EC2 and EC4 mS/cm had thicker stems and wider leaves than other treatments (Fig. 3). Plants treated with EC2 mS/cm and EC4 mS/cm were the tallest compared to other treatments (Fig. 4). The reduction of plant height, leaf size, and thickness was 12%, 26.8%, and 21.3% respectively in EC12 compared to EC2.

4. Discussion and Conclusions

Salt stressed by "onsen" water showed a significant difference (P<0.0001) on fruit soluble solids,

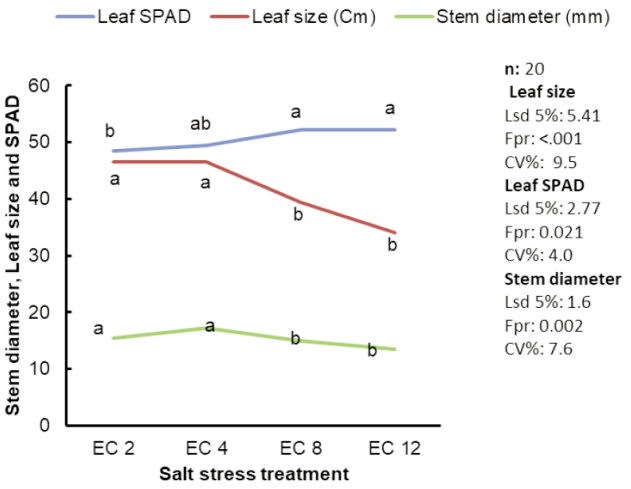

Fig. 3 - Effect of different concentration of "onsen" water on stem diameter, leaf size and SPAD of tomato plants variety 'Reika' in the greenhouse.

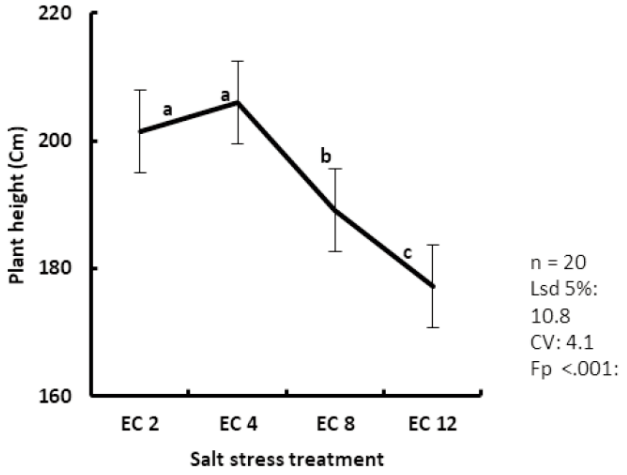

Fig. 4 - Effect of different concentration of "onsen" water on height of tomato plants variety 'Reika' in the greenhouse.

organic acid, NO_3^-concentration, and average fruit weight. Massaretto *et al.* (2018) demonstrated that soluble solids content is an important fruit quality parameter which increases depending on the salinity levels. In their study soluble solids contents were significantly higher in both Negro Yeste (60%) and Verdal (78%) landraces compared with Moneymaker (34%) tomato variety under salt stress. In general, there is a negative relationship between SSC and weight in tomato (Higashide and Heuvelink, 2009). Many studies (Adams and Ho, 1992; Cuartero and Fernández-Munaoz, 2001; Cuartero *et al*., 2006) demonstrated that one of the well-known effects of salinity is the increasing of SSC and antioxidants but with a reduction in the fruit size and weight. In present study, high EC increased the tomato fruit SSC and organic acid while weight and size declined (Fig. 1 and 2). Fruit weight and size reduction under salinity stress was triggered by inhibition of water uptake by the root resulting in insufficient amount of water required for the fruit and increased concentrations of reducing sugars and acids as compared to non-saline conditions. Thus increased concentration of soluble solids was observed in the present study (Fig. 1).

Fruit juice acidity increased with high EC and this could be due to the higher Na content in the fruit juice, since this was the main ion found in "onsen" water. Amjad *et al*. (2014) stated that the accumulation of reducing sugars and organic acids is responsible for increased titratable acidity and decreased pH of the fruit juice. The reduction in fruit water content have caused an increase in fruit dry matter content (Fig. 3). This could be as a result of drought leading to excess amounts of salt in the root zone, which leads to reduced photosynthetic capacity, or toxicity of salt in plant tissues (Amjad *et al*., 2014).

At the first truss, fruit weight was not significantly influenced by salinity. The reductions started at the second truss and this can be explained by long exposure on salinity.

Considering a reduction in fruit fresh weight (40%) compared with the increase in dry weight (50%), and SSC (50%), the lower water content in the fruits can be pointed as the main reason for the reduction in fruit weight. The accumulation of Na in the soil pot showed by the salt concentration in drained soil solution (from 0.68% to 0.9%) was due to high amount of "onsen" water applied. This contributed to the increased nutrient solution EC (from EC12 to EC16) which probably created an osmotic pressure around the roots. Thus, a reduction of water uptake by plants had resulted by NO_3^-concentration in culture medium which increased 1000 ppm to 1600 ppm.

Most of the growth parameters were affected by high salinity treatment. The plant height, stem diameter, leaf size were significantly decreased (Fig. 3 and 4). Bustomi Rosadi *et al*. (2014) mentioned that the plant height was significantly affected by high salinity due to water stress. Ashraf *et al*. (2021) also reported that tomato plants grown under salinity stress in the presence of 200 mg N kg^{-1} in different $NH4^+$:NO^{-3} ratios showed a variable decline in growth in terms of plant height, plant girth, and stem diameter compared to no salinity treatment (respective controls). The reduction in plant growth parameters of our

results can be explained by low water potential of the soil solution due to the high EC and salt concentration as it was evident in drained solution. Our results also showed an increase of fruit NO_3^- in high EC whereas fruit size and weight were reduced (Fig. 2), this can be attributed to transport of water from the roots to the fruits.The salt stress by "onsen" water in the nutrient solution reduces the ability of the plant to take up water, and this leads to reductions in the growth rate. Results of our experiment indicate that the salt stress by "onsen" water is also effective for increasing the sweetness of tomato fruit. In conclusion, the EC8 and EC12 treatments have the most effective to increase the sweetness of tomato fruit with a reduction in fruit weight.

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References

ADAMS P., 1991 - *Effects of increasing the salinity of the nutrient solution with major nutrients or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool*. - J. Hort. Sci., 66(2): 201-207.

ADAMS P., HO L.C., 1992 - *The susceptibility of modern tomato cultivars to blossom-end rot in relation to salinity*. - J. Hort. Sci., 67(6): 827-839.

AMJAD M., AKHTAR J., HAQ M.A.U., IMRAN S., JACOBSEN S.E., 2014 - *Soil and foliar application of potassium enhances fruit yield and quality of tomato under salinity*. - Turkish J. Biol., 38(2): 208-218.

ASHRAF M., NAZ U., ABID M., SHAHZAD S.M., AZIZ A., AKHTAR N., NAEEM A., MÜHLING K.H., 2021 - *Salinity resistance as a function of NH_4^+: NO_3^- ratio and its impact on yield and quality of tomato (*Solanum lycopersicum *L.).* - J. Plant Nutr. Soil Sci., 184(2): 246-254.

BALIBREA M.E., MARTÍNEZ-ANDÚJAR C., CUARTERO J., BOLARÍN M.C., PÉREZ-ALFOCEA F., 2006 - *The high fruit soluble sugar content in wild* Lycopersicon *species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism*. - Functional Plant Biology, 33(3): 279-288.

BUSTOMI ROSADI R.A., SENGE M., SUHANDY D., TUSI A., 2014 - *The effect of EC Levels of nutrient solution on the growth, yield, and quality of tomatoes* (Solanum Lycopersicum) *under the hydroponic System*. - J. Agric. Eng. Biotechnol., 2(1): 7-12.

CUARTERO J., BOLARIN M.C., ASINS M.J., MORENO V., 2006 - *Increasing salt tolerance in the tomato*. - J. Exp. Bot., 57(5): 1045-1058.

CUARTERO J., FERNÁNDEZ-MUNAOZ R., 1999 - *Tomato and salinity*. - Sci. Hort., 78: 83-125.

FAO, 2016 - *FAOSTAT*. - Food and Agriculture Organization statistics, http://www.fao.org/faostat.

GAUTIER H., LOPEZ-LAURI F., MASSOT C., MURSHED R., MARTY I., GRASSELLY D., KELLER C., SALLANON H., GENARD M., 2010 - *Impact of ripening and salinity on tomato fruit ascorbate content and enzymatic activities related to ascorbate recycling*. - Funct. Plant Sci. Biotech., 4(1): 66-75.

HIGASHIDE T., HEUVELINK E., 2009 - *Physiological and morphological changes over the past 50 years in yield components in tomato*. - J. Am. Soc. Hort. Sci., 134(4): 460-465.

HO L.C., 1999 - *The physiological basis for improving tomato fruits quality*. - Acta Horticulturae, 487: 33-40.

KAMRANI M.H., KHOSHVAGHTI H., HOSSEINNIYA H., 2013 - *Effects of salinity and hydroponic growth media on growth parameters in tomato (*Lycopersicon esculentum *Mill.)*. - Int. J. Agron. Plant Prod., 4 (10): 2694-2698.

LU J., SHAO G., CUI J., WANG X., KEABETSWE L., 2019 - *Yield, fruit quality and water use efficiency of tomato for processing under regulated deficit irrigation: A meta-analysis*. - Agric. Water Manag., *222:* 301-312.

MAGÁN J.J., GALLARDO M., THOMPSON R.B., LORENZO P., 2008 - *Effects of salinity on fruit yield and quality of tomato grown in soil-less culture in greenhouses in Mediterranean climatic conditions*. - Agric. Water Manag., 95(9): 1041-1055.

MASSARETTO I.L., ALBALADEJO I., PURGATTO E., FLORES F.B., PLASENCIA F., EGEA-FERNÁNDEZ J.M., BOLARIN M.C., EGEA I., 2018 - *Recovering tomato landraces to simultaneously improve fruit yield and nutritional quality against salt stress*. - Front. Plant Sci., 9: 1778.

OZTEKIN G.B., TUZEL Y., TUZEL I.H., 2013 - *Does mycorrhiza improve salinity tolerance in grafted plants*?. - Scientia Hort., 149: 55-60.

ROCK C., YANG W., GOODRICH-SCHNEIDER R., FENG H., 2012 - *Conventional and alternative methods for tomato peeling.* - Food Engineering Reviews, 4(1): 1-15.

SHABALA L., MACKAY A., TIAN Y., JACOBSEN S.E., ZHOU D., SHABALA S., 2012 - *Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (*Chenopodium quinoa). - Physiologia Plantarum, 146(1): 26-38.

SHANKARA N., JOEP VAN LIDT D.J., MARJA D.G., MARTIN H., BARBARA V.D., 2005 - *Cultivation of Tomato:*

Production. - Processing and Marketing, Agrodok, 17.

ZHAI Y., YANG Q., HOU M., 2015 - *The Effects of saline water drip irrigation on tomato yield, quality, and blossom-end rot incidence - A 3a case study in the South of China*. - PloS One, 10(11): e0142204.

ZHANG H., XIONG Y., HUANG G., XU X., HUANG Q., 2017 - *Effects of water stress on processing tomatoes yield, quality and water use efficiency with plastic mulched drip irrigation in sandy soil of the Hetao Irrigation District.* - Agric. Water Manag., 179: 205-214.

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Review paper

AHS
Advances in Horticultural Science

Hexachlamys edulis (Berg) Kausel & Legrand, "ubajay", a native fruit species from South America

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Key words: Ethnobotany, Myrtaceae, nutraceutical fruit.

Abstract: *Hexachlamys edulis* (Berg) Kausel & Legrand, "ubajay" is a native fruit species from South America belonging to the Myrtaceae family. Undoubtedly, it is a prominent species that provides potentially nutraceutical fruits, leaves with secondary metabolites of interest, and other organs with great benefits for human health and new alternatives for production systems. The aim of this work is to carry out a bibliographic review of all the scientific material published on this species to date. Research of this species could reveal and register its ethnobotanical potential.

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

South America occupies a prominent place with 263 underutilized fruit species, among which several of them play an important role in food and human nutrition. They have compounds that make them functional foods, have resilience to inclement weather, resist biotic stress and finally have important genes for their breeding (Brauch, 2016).

Furthermore, these species are underutilized for various reasons such as: the ignorance of their nutritional value and commercial potential, botanical misinformation, lack of promotion and popularization and the rapid disappearance of the ecosystem due to habitat destruction.

Non-traditional fruits are considered to play an important role in mitigating the problems of world food in a context with growing population and malnutrition (Nandal and Bhardwaj, 2014; Dandin and Krishna Kumar, 2016; S Ajay Vino and Sinija, 2016).

In addition, Denardin *et al*. (2015) support that there is substantial evidence of the beneficial effects of diets rich in fruits and vegetables.

There is a preference to choose the consumption of fruits with superior qualities; thus, their health benefits and perception as exotic have led to a growing demand from consumers disposed to pay higher prices than the most traditional fruits (González Vega, 2013). However, little is known about these species, and it is essential to research for being part of our economic, social and cultural heritage (Alonso and Desmarchelier, 2014).

Hexachlamys edulis (O. Berg) Kausel & D. Legrand, "ubajay" (*H. edulis*) is certainly a prominent species, distributed naturally in an important area of South America, which provides potentially nutraceutical fruits, leaves with secondary metabolites of interest, and other usable organs that provide great benefits for human health and new alternatives for production systems.

The aim of this study was to conduct a review of all scientific literature on *H. edulis* to date.

2. Materials and Methods

In order to get comprehensive information on this species, we have extensively explored available databases like Science Direct, Google Scholar, Mendeley and PubMed.

In total, we selected 55 articles through database searching with the names "*Eugenia myrcianthes*", "*Hexachlamys edulis*" and "*Myrcianthes edulis*".

Finally, the articles were used for data extraction and analysis, which were related to endemic area, systematic and phylogeny, morphological characterization, chemistry composition and ethnobotany.

3. Results

Endemic area

There are no references about *H. edulis* distribution on another continent, therefore it can be confirmed that this is an endemic species of South America. Area of collection and conservation of this species by different researchers is showed in figure 1.

Hexachlamys edulis has been studied or cited in the Argentina provinces of Entre Ríos, Corrientes, Misiones, Santa Fe, Formosa and Chaco. In Uruguay it grows spontaneously in Soriano, Río Negro, Paysandú and Artigas departments, while in Paraguay it was found in Central and Cordillera departments. Finally, in Brazil its distribution was more widespread to Rio Grande do Sul, Paraná, Santa Catarina, Mato Grosso do Sul, Minas Gerais, Sao Paulo and Goiás states.

Hexachlamys edulis has been observed in areas near water courses, where Paraná, Uruguay and Paraguay rivers are the most important. Also, it has been referred in gallery forest, delta, islands and the Paranaense jungle.

According to these studies and mentions, *H. edulis*

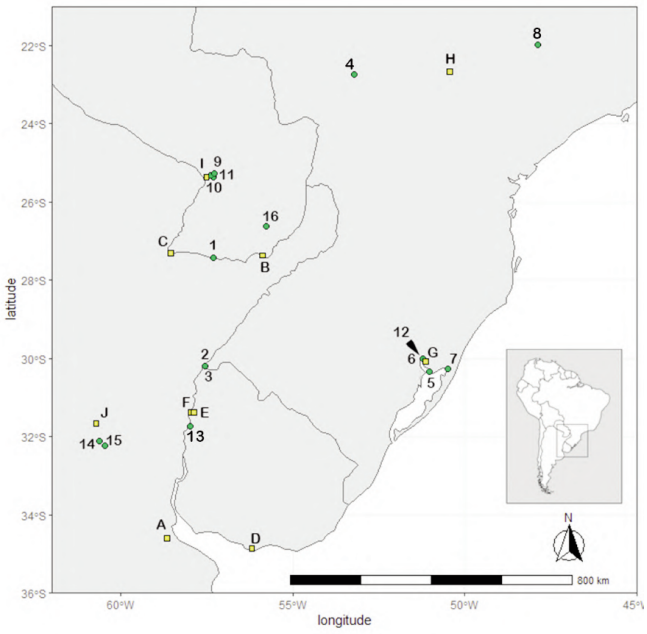

Fig. 1 - Map showing the collection sites of *Hexachlamys edulis* samples cited in this study. The circles represent samples collected from wild populations and the squares show material from herbaria or non-wild populations. Data was compiled using Microsoft Excel and R programming language (R Core Team, 2018).

References	Latitude S	Longitude W
A Molina, 2016	34°36'34.96"	58°40'31.31"
B Lorca *et al.*, 1995	27°21'53.28"	55°53'36.84"
C Franceschini, 2000	27°18'34.35"	58°33'44.03"
D Grela, 2004	34°51'32.03"	56°11'58.76"
E González, 2003	31°22'36.55"	57°58'34.22"
F Vignale and Bisio, 2005	31°23'5.84"	57°53'3.79"
G Kinupp and De Barros, 2008	30° 4'11.54"	51° 8'23.17"
H Branco *et al.*, 2016	22°39'16.36"	50°26'13.43"
I Schmeda-Hirschmann, 1995	25°21'14.12"	57°31'9.01"
J Rozycki *et al.*, 1997	31°40'8.99"	60°43'44.47"
1 Cecotto *et al.*, 2007	27°25'52.04"	57°19'25.70"
2 Cardoso Marchiori and Santos, 2010	30°12'26.71"	57°33'35.91"
3 Santos *et al.*, 2014	30°12'26.68"	57°33'33.27"
4 Romagnolo and Souza, 2004	22°45'2.92"	53°13'20.05"
5 Da Cruz, 2012	30°20'43.71"	51° 1'26.89"
6 Da Cruz, 2012	30° 3'7.35"	51°10'32.41"
7 Da Cruz, 2012	30°15'52.20"	50°29'59.84"
8 Takao *et al.*, 2015	21°58'29.68"	47°52'28.53"
9 Theoduloz *et al.*, 1988	25°21'36.36"	57°19'48.44"
10 Rodriguez *et al.*, 1992	25°18'19.62"	57°23'13.40"
11 Schmeda-Hirschmann *et al.*, 1996	25°16'0.56"	57°17'3.71"
12 Apel *et al.*, 2005	30° 0'21.60"	51°14'13.28"
13 Bertucci *et al.*, 2008	31°43'30.03"	58° 0'1.48"
14 Fagundez, 2011	32° 06' 19 6''	60°38' 25 5''
15 Fagundez, 2011	32°13'21 2"	60°27'51.4"
16 Dujak, 2015	26°36'36.09"	55°46'26.88"

is distributed at least in one million Km^2.

Systematics and phylogeny

Myrtaceae family has 131 genera and more than 4620 species (Stevens, 2017) and up to 5800 species according to Stefanello *et al*. (2011). The family is divided into two large tribes according to the fruit consistence: Leptospermeae if the fruit is dry or Myrteae if it is fleshy.

The Myrteae tribe or subfamily, contains three subtribes: Myrcinae Berg, Orthotestimoninae Berg, and Eugeniinae Berg. The latter tribe includes the genus *Hexachlamys* (Legrand, 1962) that presents approximately 10 species distributed in South America (Cruz *et al*., 2011), among which is *H. edulis*.

Authors cited the species *H. edulis* with several names and also including it in different genera (Table 1). *Psidium amygdalinum* (Hooker and Arnott, 1833) is the oldest available name for this species. Sometime later the species was named *Myrcianthes edulis* by O. Berg, and then Bentham and Hooker rename this taxon to *Eugenia*. This species became known as *Eugenia edulis* until Niedenzu showed that the name already existed and introduced *Eugenia myrcianthes* Nied. Finally, Kausel and Legrand published the new nomenclature as *Hexachlamys* (Proença, 2006).

The name *H. edulis* was first published in 1950 and since 1968 the genus *Hexachlamys* was independent (McVaugh, 1968). *Hexachlamys* was differentiated from *Eugenia* by Berg based on the number of chalice pieces and the embryo morphology. Flowers of *Eugenia* gender have tetrameric chalice while those of *Hexachlamys* have pentameric or hexameric calix, and the embryo of *Hexachlamys* have hypocotyl visible and exserted and cryptic in *Eugenia* (Da Cruz *et al*., 2013); however, some authors considered this argument inconsistent to separate the genus (Ciarlante, 2003; Da Cruz, 2012).

Other names used for the species were *Luma grisebachii, Luma myrcianthes, Myrtus excelsa, Calomyrtus excelsa, Myrcia gemmiflora* (eds), *Campomanesia cagaiteira, Myrcia sparsifolia, Hexachlamys excelsa, Eugenia montevidensis, Eugenia edulis* ex Grisebac, *Myrciaria edulis* Skeels, *Myrciaria plicatocostata* O. Berg, *Eugenia plicatocostata* Glaz, *Marlierea edulis* Nied, *Plinia anonyma* Sobral, *Plinia edulis* Vell, *Plinia plicatocostata* O. Berg Amshoff (Rotman, 1982; Borges *et al.*, 2014).

Since the publication of Mattos (1995) and Landrum and Kawasaki (1997) it was discussed whether *Hexachlamys* should be considered as a different genus or as a synonym of *Eugenia*.

In addition, new phylogenetic molecular analysis have revealed that *Hexachlamys* species do not form a monophyletic clade, so the *Hexachlamys* proposal as a synonym for *Eugenia* was corroborated (Cruz *et al*., 2011; Da Cruz, 2012; Da Cruz *et al*., 2013; Mazine *et al*., 2014, 2016, 2018).

Mazine *et al*. (2018) suggested more consistent and stable classification: *Hexachlamys* as a subgenus of *Eugenia*. This last denomination, *H. edulis*, is the most currently used as is observed in figure 2 (Proença, 2006; GBIF, 2019).

Different authors (Niedenzu, 1893; Mattos, 1995; Landrum and Kawasaki, 1997; Sobral, 2003; Proença, 2006; Da Cruz, 2012; Mazine *et al*., 2014, 2016;

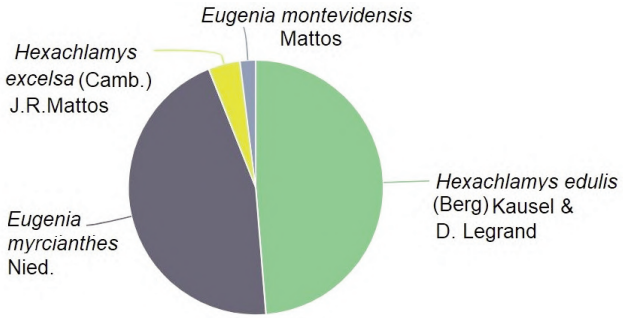

Fig. 2 - Different names used to classify *Hexachlamys edulis*. Figure taken from GBIF (2019).

Table 1 - Different names assigned to the species by botanists over the years

Year	Botanists				
	Hooker & Arnott	O. Berg	Bentham & Hooker	Niedenzu	Kausel & Legrand
1833	*Psidium amygdalinum*				
1857		*Myrcianthes edulis*			
1865			*Eugenia edulis*		
1893				*Eugenia myrcianthes*	
1950					*Hexachlamys edulis*

WCSP, 2020) argue that it is correct to accept the synonymy between *H. edulis* (Berg) Kausel & Legrand and *Eugenia myrcianthes* Nied.

Morphological characterization

Hexachlamys edulis is a fruit tree with globular treetop (Fig. 3A), with an altitude up to 10 meters according to Rotman (1982), 12 meters according to Legrand and Klein (1977), Cardoso Marchiori and Santos (2010) and 15 meters by Dematté (1997).

Persistence of the foliage is contradictory (Carrere, 2008). Rotman (1982) consider this species as evergreen but in Uruguay it shows leaf fall from late May to late August and blooming at the beginning of the spring without leaves. Foliage unfolds later, when the anthesis declines and vegetative growth stops in early December (González, 2003). A more exhaustive observation allowed determining that this species is phanerophytes - by the aerial location of its buds (Raunkiaer, 1934) - and deciduous with concomitant foliar renewal and that apparently low temperatures accelerate defoliation (González, 2003).

Leaves are described as petiolated, with a coriaceous leaf blade, pubescent, oval or elliptic-oblong, briefly acuminate, obtuse base, (3-) 5-9 cm of length by (1-) 2.5-3.4 cm, reticulate venation, with the median nerve prominent on the abaxial face; pubescent

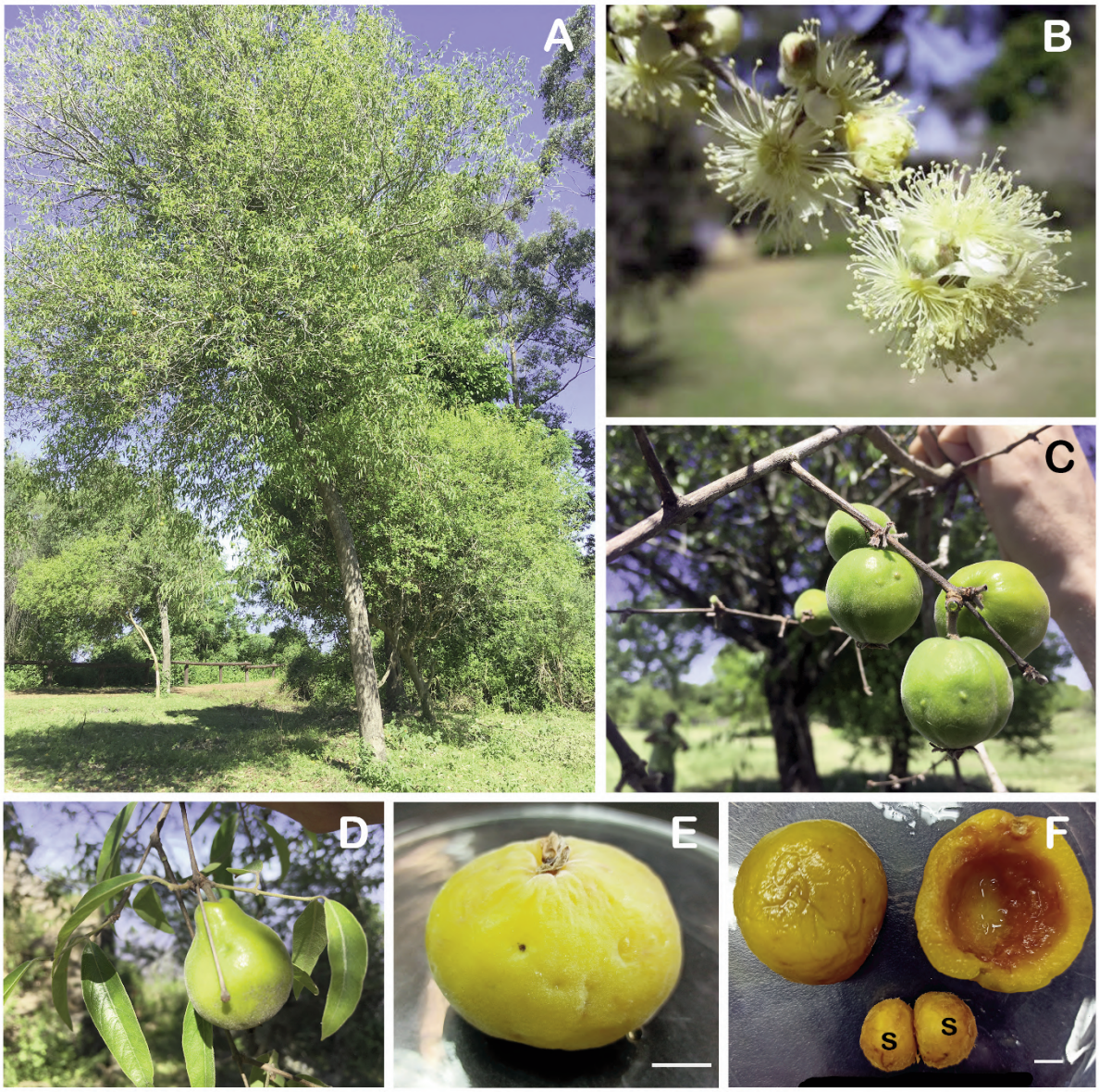

Fig. 3 - *Hexachlamys edulis* grown in Argentina. (A) tree grown in Parque Nacional El Palmar, Entre Ríos; (B) cluster of flowers; (C-D) inmature fruit; (C) globose; (D) piriform; (E-F) mature fruit; (F) disected fruit with seeds (s). Bars = 1 cm.

petioles, (3-) 7-10 mm of length (Rotman, 1982). Lorca *et al*. (1995) described polygonal to sinuous cells of various sizes and sparse trichomes stand out in the adaxial epidermis; the cuticle is thick and striated. In their abaxial epidermis they describe abundant single celled trichomes and clustered and raised stomata. Stomata are paracytic, that is, they have 2 adjoining cells arranged parallel to the occlusive cells. Also, the presence of hypodermis towards the adaxial face, palisade parenchyma 1-stratified and spongy parenchyma 5-6-stratified, with large intercellular spaces stand out. The central rib has flabeliform xylem and phloem in bundles located on the abaxial and adaxial sides of the xylem. Sclerenchymal sheath interrupted on the sides. Collenchyma present on the abaxial side and 3-4 hypodermis stratified on the adaxial side.

Flowers are typical of Myrtaceae; appear in the leaf axils in apical position, are white, solitary and hermaphroditic (Fig. 3B). Rotman (1982) describes clusters with 2-4 flowers with pubescent pedicels from 1 to 22 mm in length, puberulous, linear lanceolate, up to 5 mm in length; pubescent hypanthus; sepals (4-) 5, puberulous, deltoid, acute, persistent, 3-5 mm in length; petals (4-) 5, obtuse, ciliolate, pubescent on the outer, face spacedly hairy on the inner face, 5-9 mm length; stamens 5-9 mm in length, pubescent staminal disc; 2-3 locular ovary with axillary placentation. Although there are no morphological references of nectariferous glands, Fagundez (2011) cites *H. edulis* as a native pollen-nectariferous species of interest.

Flowering season is variable according to diferent the researchers. Flowering, occurs from August to January in northeastern Argentina, in the department of Artigas, Uruguay, and in Asunción, Paraguay by Rotman (1982). Instead, for Dematté (1997) it happens between August and November, that is, it reduces the range, without specifying the location, while Vignale and Bisio (2005) reports a shorter flowering period, during September, in Salto, Uruguay. In the same locality, González (2003) observes that in early October flowering declines and ends entirely in the middle of the month. For plants grown in the locality of Lajeado, Brazil, Guizzo and Jasper (2005) recorded flowering from August to September. Finally, Fagundez (2011) found that *H. edulis* reaches full bloom during the month of October in Diamante, Argentina.

Fruit is a globose drupe (Lughadha and Proenca, 1996) with a yellow color, about 5 cm in maximum equatorial diameter (Lorenzi *et al*., 2000) and up to 50 g of fresh weight in the mature state. Fruit set and ripening occur in a few weeks, staggered from mid October to the end of November when the harvest can be done (Vignale and Bisio, 2005). Rotman (1982) describes an edible fruit, globose or piriform (Fig. 3C-D), up to 9 cm long. Fruits are yellow, up to 4 cm in diameter according to González (2003). It is a barely fluffy fruit, with an orange flesh, very juicy, with a slightly sour taste and a pungent odor when fully ripe (www.descubriendocorrientes2012.com) (Fig. 3E-F). Also, it has been described as sweet sour, pleasant, and a quickly maturing (González, 2003) and as a very acidic fruit (Chebez and Masariche, 2010). However, other authors have characterized the ripe fruit by a disgusting smell, although it would seem that there is great diversity in the fruits between genotypes (Vignale and Bisio, 2005), to which Carrere (2008) attributes that more or less fruits could be found pleasant to smell depending on the subjectivity of those who perceive it.

Regarding to associated pathologies, González (2003) found that most of the fruits had insect bites, but without specifications; only Rossini *et al*. (2015) cites *H. edulis* as host of *Anastrepha fraterculus* (Wiedemann).

The seed is exalbuminated, globose and during germination it remains enclosed by a woody structure that some authors interpreted as endocarp (Rotman, 1982) (Fig. 3F); but it has not been anatomically corroborated (Franceschini, 2000). It has a single embryo (Lughadha and Proenca, 1996) solid, globose and glabrous (Franceschini, 2000) with fully welded cotyledons. Germination is hypogeal and cryptocotilar.

The seedling is completely covered with simple hairs, undeveloped hypocotyl, light green and cylindrical epicotyl. Alternate membranous cataphylls have acute apex, obtuse base and entire margin. Simple, alternate or opposite nomophiles; petiole ribbed on adaxial face; elliptical blade, chartaceous, light green, sharp apex and base and entire margin (Franceschini, 2000).

Chemistry composition

Researchers promote the consumption of diets rich in fruits and vegetables due to its health beneficial effects, such as lowering the risk of cardiovascular disease, cancer and conditions associated with aging and oxidative stress (Gomez da Silva *et al*., 2019). Studies show that these foods can prevent dis-

eases and disorders due to the presence of bioactive compounds with antioxidant properties. These compounds interfere with biological mechanisms such as the protection against free radicals, cellular signaling mediated for free radicals, inflammation, allergies, platelet aggregation, ulcers, viruses, tumors and hepatotoxicity (Denardin *et al*., 2015).

Several studies have shown that the fruits of Myrtaceae have antioxidant activity (Borges *et al*., 2014) due to the presence of anthocyanins, flavonoids, carotenoids or other secondary metabolites.

In particular, *H. edulis* fruit would have great potential as a functional food due to its compositional profile, outstanding nutritional value (Vignale and Bisio, 2005), very favorable for health, and considering that its vitamin content has been highlighted (Cecotto *et al*., 2007). In addition, fruit has vitamin C quantified in 75.1 mg/100 g of fresh matter (Rozycki *et al*., 1997). Also, different phenolic compounds (2181.42 mg GAE/100 g of dry matter), carotenoids (242.53 µg/g of dry matter) and antioxidant activity (153.09 µmol Trolox Equivalent/g of dry matter) have been quantified (Branco *et al*., 2016). It also stands out for its protein content of 8.05% in dry matter, and good relative amounts of Zinc and Boron minerals (Kinupp and De Barros, 2008).

Hexachlamys edulis was noted for its high content of total pectin (403.5 mg/100 g) in fresh fruits tissue (Rozycki *et al*., 1997). This high content explains the excellent quality of the fruit jelly of this species cited by Kinupp and De Barros (2008).

All these data show the potential of this fruit tree, especially for the fruit pulp, juice and jelly agroindustry. However, this species is not considered yet by Brazilian fruit producers and researchers (Kinupp and De Barros, 2008), unlike the manifest interest observed in other countries of South America.

On the other hand, the leaf of the species belonging to the Myrtaceae is the most used plant organ in South America for medicinal purposes (Camelo Munevar, 2016), and perhaps for this reason, the chemical composition of the leaves has been the most studied. *H. edulis* leaves contain steroid triterpenes, low molecular weight terpenes, tannins, polyphenols, saponins, alkaloids, flavonoids, and glycosides (Bertucci *et al*., 2008). Flavonoid glycosides: myricetin 3-O-rhamnoside; myrcetin 3-O-pentoside; quercetin 3-O-pentoside; quercetin 3-O-rhamnoside and myricetin deoxyhexoside-gallate (Schmeda-Hirschmann, 1995; Schmeda-Hirschmann *et al*., 1996; Celli *et al*., 2010; Takao *et al*., 2015). Essential oils have a predominance of sesquiterpenes, of which β-selinen (16.1%), β-caryophyllene (8.3%) and δ-cadinene (8.3%) are the most abundant (Apel *et al*., 2005). Also, Borges *et al*. (2014) found β-caryophyllene, its oxide and caryophyllene oxide (39.3%).

Ethnobotany

According to Legrand and Klein (1977), *H. edulis* is a species frequently cultivated in Rio Grande do Sul (Brazil), for its tasty and fragrant fruits. Fruits are consumed fresh, in jams, juices, jellies (Da Cruz, 2012) and ice creams have been made with great acceptance. Kozel (1991) describes that consumption of *H. edulis* fruits favor fights bladder stones and nephritic stones. Also, two Mbyá Guaraní communities of the San Rafael Park Reserve harvest the fruit for food (Dujak *et al*., 2015). The fruit is considered by some people to be slightly laxative (Lorenzi *et al*., 2000).

Leaves are widely used as infusions in Brazil for the treatment of bronchitis, cough, whooping cough (Camelo Munevar, 2016) and diabetes (Rodriguez *et al*., 1992). *H. edulis* leaf extract causes a significant response in blood glucose levels due to its rich content of flavonoids and tannins. In addition, Lorca *et al*. (1995), highlight its use for the treatment of diabetes in the form of herbal teas or with *mate*. Consumption was reaffirmed by Pirondo *et al*. (2011), due to the supply of the product in the markets of Corrientes, Argentina. Other authors highlighted the benefits of the leaves and their medical use to reduce uric acid due to the inhibition of the enzyme xanthine oxidase, producer of uricate, whose excess is considered to cause hyperuricemia in gout (a form of inflammatory arthritis) (Lio *et al*., 1985). Theoduluz *et al*. (1988) analyzed *H. edulis* leaves and it was proved to be the most active among the 15 species in a comparative study.

Other possible secondary uses are the production of vinegar from the fruit (Carrere, 2008) or production of essential oils from its flowers (Cecotto *et al*., 2007). Also, its wood is useful (Molina, 2016) due to it is moderately heavy, hard, compact and resistant, suitable for common carpentry, tool handles, turning (Cardoso Marchiori and Santos, 2010) and its bark can be used in the chemical industry for the preparation of white ink and tannins (Carrere, 2008). It is used as alternative forage in Paraguarí, Paraguay (Benítez and Bertoni, 2015) or can also be used for reestablishing native woodland or forest gardens

(Lorenzi, 2002). Chebez and Masariche (2010) includes *H. edulis* in their list of healing trees.

H. edulis is known by many vernacular names such as "ubajay" and due to its similarity to the peach (*Prunus persica* L. Batsch.), it is called "duraznero de monte" (in Argentina and Uruguay) (Hanelt *et al*., 2001). Also, *H. edulis* was named "iba hay", "ibajai", "igu jhay mi", "uva jy" (Legrand, 1941; Rotman, 1982) and "Clagye locoic" in Mocovic lenguage (Rosso and Scarpa, 2012). In Brazil it is known as "cereja do rio grande", "ocorocil lo", "cerejeira", "ivai", "pessegueiro bravo", "pêssego do mato", "ubajaí" and "ibajaí" (Romagnolo and Souza, 2004; Proença, 2006). In Paraguay it is known as "yvahái" (Dujak *et al*., 2015).

4. Conclusions

Despite the ecological and economic importance, little is known about this species of the genus *Hexachlamys*, so it is important to revalue its use. *H. edulis* (O. Berg) Kausel & D. Legrand, "ubajay" research could reveal and record its ethnobotanical potential based on the chemical composition of its fruits and other organs. At the same time, we refer to a species with the possibility of cultivation that offers the opportunity to diversify production, in this case, for farmers on the Argentine coast, southern Brazil, Paraguay, western Uruguay, and why not in a future, in other regions.

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References

ALONSO J., DESMARCHELIER C., 2014 - *Plantas medicinales autóctonas de la Argentina: bases científicas para su aplicación en atención primaria de la salud.* - 1ed. Corpus Libros Médicos y Científicos, CABA, Argentina.

APEL M.A., SOBRAL M., MENUT C., BASSIERE J.M., ZUANAZZI J.Á., SCHAPOVAL E.E.S., 2005 - *Volatil constituents of four* Hexachlamys *species growing in South Brazil.* - Flavour Fragance Journal, 20: 176-179.

BENÍTEZ B., BERTONI S., 2015 - *Diversidad y homogeneidad de especies arbóreas y arbustivas utilizadas como forrajeras alternativas en área de influencia del Arroyo Caañabé, Departamento Paraguarí-Paraguay*. - Steviana, 7: 57-73.

BERTUCCI A., HARETCHE F., OLIVARO C., VÁZQUEZ A., 2008 - *Prospección química del bosque en galería de río Uruguay.* - Revista Brasileira de Farmacognosia, 18(1): 21-25.

BORGES L.L., CARDOSO CONCEIÇÃO E., SILVEIRA D., 2014 - *Active compounds and medicinal properties of* Myrciaria *genus.* - Food Chemistry, 153: 224-233.

BRANCO I.G., KIKUCHI T.T., SANJINEZ ARGANDONA E.J., FREITAS MORAES I.C., HAMINIUK C.W.I., 2016 - *Drying kinetics and quality of uvaia (*H. edulis*) powder obtained by foam-mat drying*. - Int. Jof Food Sci. Techn., 51: 1703-1710.

BRAUCH J.E., 2016 - *Underutilized fruits and vegetables as potential novel pigment sources*. - Handbook on Natural Pigments in Food and Beverages, pp. 305-335.

CAMELO MUNEVAR D.O., 2016 - *Contribución al estudio fitoquímico de frutos de* Syzygium paniculatum G. *y evaluación de su actividad antioxidante*. - Undergraduate thesis, Universidad Francisco José de Caldas, Bogotá, Colombia.

CARDOSO MARCHIORI J.N., SANTOS S.R. DOS, 2010 - *Anatomía das madeiras de* campomanesiaaurea *O. Berg e* Eugenia myrcianthes *Niedenzu (Myrtaceae).* - Balduinia, 22: 23-30.

CARRERE R., 2008 - *El ubajai (*Hexachlamis edulis*): un árbol frutal indígena*. - https://www.guayubira.org.uy/monte/Ubajai.pdf.

CECOTTO J.A., TAIARIOL D.R., CÁCERES S., 2007 - *Colección de frutos tropicales de la EEA INTA Bella Vista*. - Publicación Técnica, no. 21.

CELLI G.B., PEREIRA NETTO A.B., BETA T., 2010 - *Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of brazilian cherry (*Eugenia uniflora *L.) throughout the fruit developmental stages.* - Food Research International, 44: 2442-2451.

CHEBEZ J.C., MASARICHE M., 2010 - *Trees of Argentina*. - Albatros, Buenos Aires, Argentina, pp. 128.

CIARLANTE C.S., 2003 - *Sinópsis de géneros y taxones supragenéricos de Argentina*. - http://www. floraargentina.edu.ar/wp-content/uploads/2019/05/80-MYRTACEAE,parte3.pdf.

CRUZ F., TURCHETTO ZOLET A., VETO N., MONDIN C., ALMERÃO M., MARGIS R., 2011 - *Molecular phylogenetics of the genus* Hexachlamys *(Myrtaceae) using chloroplast and nuclear markers*. - BMC Proceedings, 5(7): 6.

DA CRUZ F., 2012 - *Sistemática e filogenética molecular do genero* Hexachlamys *(Myrtaceae) a través do uso de marcadores plastidiais e nucleares.* - PhD Thesis, Universidad Fêderal do Rio Grande, Rio Grande, Brazil,

pp. 71

DA CRUZ F., TURCHETTO ZOLET A.C., VETO N., MONDIN C.A., SOBRAL M., ALMERÃO M., 2013 - *Phylogenetic analysis of the genus* Hexachlamys *(Myrtaceae) based on plastid and nuclear DNA sequences and their taxonomic implications*. - Botanical Journal of the Linnean Society, 172(4): 532-543.

DANDIN S.B., KRISHNA KUMAR N.K., 2016 - *Neglected and underutilized fruit species: an insurance against global mal and under nutrition.* - http://www.fao.org/ fileadmin/templates/rap/files/meetings/2016/161203_session1_4.pdf.

DEMATTÉ M.E.R.P., 1997 - *Ornamental use of Brazilian Myrtaceae*. - Acta Horticulturae, 452: 143-180.

DENARDIN C.C., HIRSCH G.E., DA ROCHA R.F., VIZZOTTO M., HENRIQUES A.T., MOREIRA J.C.F., GUMA F.T.C.R., EMANUELLI T., 2015 - *Antioxidant capacity and bioactive compounds of four Brazilian native fruits.* - J. Food Drug Analysis, 23(3): 387-398.

DUJAK M.M., FERRUCCI S., VERA JIMÉNEZ M., PINEDA J., CHAPARRO E., BRÍTEZ M., 2015 - *Registros sobre las especies vegetales alimenticias utilizadas por dos comunidades indígenas Mbyá - Guaraní de la Reserva para Parque Nacional San Rafael, Itapúa - Paraguay*. - Steviana, 7: 25-47.

FAGUNDEZ G.A., 2011 - *Estudio de los recursos nectaríferos y poliníferos utilizados por* Apis mellifera L*. en diferentes ecosistemas del Departamento Diamante* (Entre Ríos, Argentina). - PhD Thesis, Universidad Nacional del Sur, Bahía Blanca, Argentina.

FRANCESCHINI M.C., 2000 - *Morfología de embriones y plántulas de especies leñosas del nordeste argentino*. - Bonplandia, 10(1-4): 143-154.

GBIF, 2019 - *Catalogue of Life. Species 2000 & ITIS Catalogue of Life* - https://www.gbif.org/es/species/5418198.

GOMES DA SILVA A.P., SPRICIGO P.C., PURGATTO E., ALENCAR S.M., SARTORI S.F., JACOMINO A.P., 2019 - *Chemical composition, nutritional value and bioactive compounds in six uvaia accessions*. - Food Chemistry, 294: 547-556.

GONZÁLEZ S., 2003 - *Ritmos de follaje y floración en algunas plantas leñosas nativas*. - Agrociencia, 7(2): 27-38.

GONZÁLEZ VEGA M.E., 2013 - *Chirimoya (*Annona cherimola *Miller), frutal tropical y subtropical de valores promisorios*. - Cultivos Tropicales, 34(3): 52-63.

GRELA I., 2004 - *Geografía florística de las especies arbóreas de Uruguay: propuesta para la delimitación de dendrofloras.* - MSc. Thesis, Universidad de la República, Montevideo, Uruguay.

GUIZZO D.J., JASPER A., 2005 - *Levantamiento das espécies arbóreas dos passeios das vias públicas do bairro americano de lajeado - RS, com indicação de solução de problemas já existentes pesquisas*. - Botânica, 56: 185-208.

HANELT P., BUTTNER R., MANSFELD R., 2001 - *Mansfeld´s encyclopedia of agricultural and horticultural crops*. - 1 ed. Springer, Berlín, Germany.

HOOKER W.J., ARNOTT G., 1833 - *Botanical miscellany*. - London 3, 317. - https://biodiversity library.org/page /779258#page/318/mode/1up.

KINUPP V.F., DE BARROS I.B.I., 2008 - *Protein and mineral contents of native species, potential vegetables, and fruits*. - Ciencia e Tecnologia de Alimentos, 28(4): 846-857.

KOZEL C., 1991 - *Guia de medicina natural*. - Plantas Medicinales, 2: 495.

LANDRUM L.R., KAWASAKI M.L., 1997 - *The genera of* Myrtaceae *in Brazil: an illustrated synoptic treatment and identification keys*. - Brittonia, 49: 508-536.

LEGRAND D., 1941 - *Lista preliminar de las mirtáceas argentinas*. - Darwiniana, 5: 463-486.

LEGRAND D., 1962 - *Lista actual de las mirtáceas de Argentina*. - Boletín de la Sociedad Argentina de Botánica, X(1): 46-51.

LEGRAND D., KLEIN R.M., 1977 - *Mirtáceas:* Campo-manesia, Feijoa, Britoa, Myrrhinium, Hexachlamys, Siphoneugena, Myrcianthes, Neomitranthes, Psidium. - In: REITZ P.R. (ed.) *Flora Ilustrada Catarinense*. Herbário Barbosa Rodrigues, Itajaí, pp. 571-730.

LIO M., MORIYAMA A., MATSUMOTOA Y., TAKAKIA N., FUKUMOTOA M., 1985 - *Inhibition of Xanthine oxidase by flavonoids*. - Agric. Biol. Chem., 49(7): 2173-2176.

LORCA G.G., AMAT A.G., GONZÁLEZ C. 1995 - *Análisis comparativo de caracteres diagnósticos para la Identificación de tres especies Argentinas de m*yrtaceae *empleadas en la medicina popular.* - Acta Farm. Bonaerense, 14(2): 81-86.

LORENZI H., 2002 - *Brazilian trees.* - Publisher Instituto Plantarum De Estudos Da Flora, Brazil.

LORENZI H., BACHER L., LACERDA, M., SARTORI S., 2000 - *Brazilian fruits and cultivated exotics publication.* - Instituto Plantarum De Estudios Da Flora, LTDA, Brazil.

LUGHADHA E.M., PROENCA C., 1996 - *A survey of the reproductive biology of the myrtoideae (Myrtaceae).* - Annals of the Missouri Botanical Garden, 83(4): 480-503.

MATTOS J.R., 1995 - *Novidades taxonômicas em Myrtaceae IX*. - Loefgrenia, 105: 1-3.

MAZINE F.F., 2016 - *Sections in* Eugenia *(Myrteae, Myrtaceae): nomenclatural notes and a key.* - Phytotaxa, 289(3): 225-236.

MAZINE F.F., QUINTINO FARIA J.E., GIARETTA A., VASCONCELOS T., FOREST F., LUCAS E., 2018 - *Phylogeny and biogeography of the hyper-diverse genus* Eugenia *(Myrtaceae: Myrteae), with emphasis on* E. *sect. Umbellatae, the most unmanageable clade*. - Taxon, 67(4): 752-769.

MAZINE F.F., SOUZA V.C., SOBRAL M., FOREST F., LUCAS E., 2014 - *A preliminary phylogenetic analysis of* Eugenia *(Myrtaceae: Myrteae), with a focus on Neotropical species*. - Kew Bulletin, 69: 94-97.

MCVAUGH, 1968 - *The genera of American Myrtaceae: An interim report.* - Taxon, 17: 354-418.

MOLINA A.M., 2016 - *El Jardín Botánico Arturo E. Ragonese (JBAER): miradas a través del tiempo, realidad y prospectiva.* - 1ed. Ediciones INTA, BA, Argentina.

NANDAL U., BHARDWAJ R.L., 2014 - *The role of underutilized fruits in nutritional and economic security of tribals: a review.* - Crit. Rev. Food Sci. Nutr., 54(7): 880-890.

NIEDENZU F., 1893 - *Myrtaceae.* - In: PRANTL K., and A. ENGLER (eds.) *Die Natürlichen Pflanzenfamilien,* 3: 57-105.

PIRONDO A., COULLERI J.P., KELLER H.Á., FERRUCCI M.S., 2011 - *External factors influence the marketing of medicinal plants in an urban environment: the case of creoles and indigenous vendors in Corrientes, Argentina.* - Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10(6): 553-569.

PROENÇA C.E.B., 2006 - *Proposal to conserve the name* Myrcianthes edulis *against* Psidium amygdalinum *(Myrtaceae)*. - Taxon, 55(2): 536-537.

R CORE TEAM, 2018 - *R: A Language and Environment for Statistical Computing.* - R Foundation for Statistical Computing. Vienna, Austria.

RAUNKIAER, 1934 - *The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer.* - Oxford University Press, London, UK, pp. 632.

RODRIGUEZ J., LOYOLA J., SCHMEDA-HIRSCHMANN G., 1992 - *Hypoglycaemic activity of* Hexachlamys edulis *('Yvahai') extract in Rats*. - Phytotherapy Res., 6: 47-49.

ROMAGNOLO M.B., SOUZA M., 2004 - *Os gêneros* Calycorectes *O. Berg,* Hexachlamys *O. Berg,* Myrcianthes *O. Berg,* Myrciaria *O. Berg e* Plinia *L. (Myrtaceae) na planície alagável do alto rio Paraná, Brasil*. - Acta Bot. Bras., 18(3): 613-627.

ROSSINI M.N., DUMMEL D.M., AGOSTINI J.P., 2015 - *Plagas cuarentenarias de frutales de la República Argentina Avances en los resultados*. - 1ed. Ediciones INTA. Allen, RN, Argentina.

ROSSO C.N., SCARPA G.F., 2012 - *Identificaciones botánicas de las plantas empledas entre los Mocovíes en la Reducción San Javier durante el siglo XVIII a partir de la obra de Florián Paucke.* - Etnobotánica en zonas áridas y semiáridas del Cono Sur de Sudamérica. Edición CEFYBO-CONICET, Buenos Aires, Argentina.

ROTMAN A.D., 1982 - *Los géneros* Calycorectes, Hexachlamys, Myrciaaria, Paramyrciaria, Plinia *y* Siphoneugena *en la flora argentina (Myrtaceaea)*. - Darwiniana, 24(1-4): 157-185.

ROZYCKI V.R., BAIGORRIA C.M., FREYRE M.R., BERNARD C.M., ZANNIER M.S., CHARPENTIER M., 1997 - *Composición de nutrientes en especies vegetales autóctonas de la región Chaqueña, Argentina*. - Archivos Latinoamericanos de Nutrición, 47(3).

S AJAY VINO H., SINIJA V.R., 2016 - *Underutilized fruits in Indian*. - Indian Food Industry Mag, 35(2): 45-46.

SANTOS S.R., CARDOSO MARCHIORI J.N., SIEGLOCH A.M., 2014 - *Diversidade estrutural em* Eugenia L. *(Myrtaceae)*. - Ciência Florestal, 24(3): 785-792.

SCHMEDA-HIRSCHMANN G., 1995 - *Flavonoids from* Calycorectes*,* Campomanesia*,* Eugenia *and* Hexachlamys *species.* - Fitoterapia, 66(4): 373-374.

SCHMEDA-HIRSCHMANN G., ZÚÑIGA J., DUTRA BEHRENS M., HABERMEHL G., 1996 - *Xanthine Oxidase inhibitory activity of flavonoids and tannins from* Hexachlamys edulis *(Myrtaceae).* - Phytotherapy Res., 10(3): 260-262.

SOBRAL M., 2003 - *A família Myrtaceae no Rio Grande do Sul.* - 1ed. Unisinos Press, São Leopoldo, RGS, Brasil.

STEFANELLO M.E.A., PASCOAL A.C.R.F., SALVADOR M.J., 2011 - *Essential oils from neotropical Myrtaceae: Chemical diversity and biological properties.* - Chemistry and Biodiversity, 8: 73-94.

STEVENS P.F., 2017 - *Angiosperm phylogeny website*. - http://www.mobot.org/MOBOT/research/APweb/.

TAKAO L.K., IMATOMI M., GUALTIERI S.C.J. 2015 - *Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado (Brazilian Savanna).* - Braz. J. Biol., 75(4): 948-952.

THEODULOZ C., FRANCO L., FERRO E., SCHMEDA-HIRSCHMANN G., 1998 - *Xanthine oxidase inhibitory activity of paraguayan myrtaceae*. - J. Ethnopharm., 24(2-3): 179-183.

VIGNALE B., BISIO L., 2005 - *Selección de frutales nativos en Uruguay*. - Agrociencia, 9(1-2), 41- 51.

WCSP, 2020 - *World checklist of selected plant families*. - http://www.kew.org/wcsp/ myrtaceae.

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Short note

AHS
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Strigolactone analogue GR24 reduces axillary bud out break and growth in tea tree, *Melaleuca alternifolia* (Maiden & Betche) Cheel

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Key words: auxin, axillary bud release, shoot architecture, tea tree.

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Strigolactone acts with other plant hormones to influence shoot architecture by suppressing axillary bud outgrowth. The exogenous application of synthetic analogues of strigolactone, such as GR24, have been investigated as a way to manage plant architecture in a number of crops. In this study we test whether GR24 can be used to supress bud outgrowth in clonal propagules of tea tree (*Melaleuca alternifolia)* in order to retain a "single stem" form desirable for machine planting. GR24 was applied to decapitated rooted cuttings of tea tree at two rates (0.5 mg L^{-1} and 1.5 mg L^{-1}), with and without auxin. By 21 days post -treatment, GR24 at both rates had significantly ($p<0.05$), reduced the mean number of axillary buds (5.7±0.4 and 5.5±0.3 buds respectively) compared to decapitated untreated control plants (8.9±0.6 buds). Suppression of buds was significantly higher again when auxin was applied in conjunction with GR24. Nonetheless, no exogenous hormone treatment was as effective at suppressing bud outgrowth as the apical dominance that occurred in intact control plants (1.1±0.4 buds).

1. Introduction

Tea tree, *Melaleuca alternifolia* (Maiden & Betche) Cheel, is a small tree, native to the subtropics of eastern Australia (ANPSA, 2012), which is cultivated in plantations for the production of a medicinally valuable essential oil (Carson *et al.,* 2006). The Australian industry has developed around improved cultivars released as seed lines, but there is recognition that clonal deployment should offer advantages in plantation uniformity and productivity (Doran *et al*., 1997). Although tea tree has been regarded as relatively easy to propagate by cloning (Shepherd *et al.,* 2013), the production of high quality propagules with the desired form compatible with automated machine planters remains a challenge.

Tea tree tip cuttings with single stem architecture suitable for mechanical planting can be produced if the apical bud remains intact (Lowe *et al.*, 2019). Nodal cuttings (apical tips removed) are preferred to tip cuttings by

some propagators for their robustness in the nursery but these tend to have a multi-stem habit which is unsuitable for machine planting as the "bushy" shoots get stuck in the delivery tubes of planters. Form-pruning of propagules prior to planting may be one way to overcome this compatibility issue, but this is labour intensive and costly on a large scale.

Shoot architecture is a consequence of complex interplay of interacting plant hormonal signals involving auxin, cytokinin (CK) and strigolactone (SL) that regulate bud release and/or subsequent growth (Ferguson and Beveridge, 2009; Leyser, 2009). Auxin is produced in the shoot apex and is actively transported down the stem, restricting bud release. Auxin does not enter the axillary buds but indirectly impacts on bud release by inhibiting auxin export from axillary buds and by regulating stem CK and SL to either promote or suppress branching, respectively (Gomez-Roldan *et al.,* 2008; Muller and Leyser, 2011; Dun *et al.,* 2012).

The influence of SL upon plant architecture was unravelled with the aid of studies on mutants with increased branching, including the ramosus (rms) mutant in pea (*Pisum sativum* L.), and the more axillary growth (max) mutant in Arabidopsis (*Arabidopsis thaliana* L.) (Brewer *et al.*, 2009; Dun *et al.,* 2013). Mutants have exaggerated branching habits relative to wild type plants because they are deficient in SL due to genetic changes in transcription or hormonal pathways (Umehara *et al.,* 2008; Yaish *et al.,* 2010). Potential horticultural applications of SL include, management of plant architecture and control of fruit ripening, although high cost of SL production and regulatory approvals remain a limitation to large scale commercial use (Vurro *et al.,* 2016; Ferrero *et al.,* 2018).

In this study we investigated the use of exogenous hormones to manage shoot form of tea tree cuttings during propagation. The aim was to test whether application of an exogenous synthetic SL, GR24, or a combination of GR24 and auxin, can suppress axillary bud release in tea tree. Suppression by SL was studied in decapitated plants where axillary bud outgrowth was triggered by removal of the apical bud.

2. Materials and Methods

Plant growth environment and reagents

Experiments were conducted at Southern Cross University, Lismore Campus, NSW, Australia during 2020, inside controlled environment growth cabinets, under 16/8 hour photoperiod, and with temperature set at 26°C. Stock hormone solutions were prepared by following Brewer *et al*. (2009) for indole -3 acetic acid (IAA), and Manandhar (2016) for GR24. Working solutions of 1.75 mg L^{-1} (10 µM) for IAA, and 0.5 mg L^{-1} (1.68 µM), and 1.5 mg L^{-1} (5 µM) for GR24, were prepared by dilution with distilled water and stored at 4°C.

Method and reagent validation using pea plants

Initial tests were carried out using the *P. sativum* type *rms1* mutant (provided by Professor C. Beveridge, University of Queensland, Australia) to confirm competency of reagents and methods in our laboratory. Testing of mutant pea followed methods of Manandhar, (2016). Application of 0.3 mg L^{-1} and 1 mg L^{-1} GR24 to the *rms1* mutant of pea significantly reduced side branching compared to the untreated *rms1* control, thus validating the method in our laboratory.

Testing GR24 applications on tea tree

The experiment used a Randomised Complete Block Design, with four blocks (replicates) of five plants in line plots, subject to one of six treatments (four hormone or two control treatments, giving a total of 120 plants). Plants subject to hormone treatments either had a low or high treatment of GR24 (0.5 mg L^{-1} or 1.5 mg L^{-1}) with or without supplemental IAA (1.75 mg L^{-1}). Control treatments consisted of decapitated plants, and plants with an intact apex in distilled water.

Scion from a clonal line was set in January 2020 using the mini cutting technique with intact apical buds (Lowe *et al*., 2019). The GR24 experiments were conducted 5 months post-setting when cuttings had rooted and possessed a single main stem with no visible axillary buds detectable with a 10x magnification hand lens (Fig. 1 left). Roots were washed free of media and trimmed to 5 cm in length. Except for control plants, the shoot apex was removed (approximately 2 cm) above node 35 (counted basipetaly from the lowest detectable node) to provoke release from apical dominance (Thimann and Skoog, 1934). All plants were then positioned upright in clear 50 mL Falcon tubes containing 5 mL of treatment solution, or distilled water, so that only the roots were in contact with treatment solutions (Fig. 1 middle).

The experiment was conducted over 21 days in June 2020. Treatment solutions were applied on day 1 and replaced every 7 days. For plants subjected to a

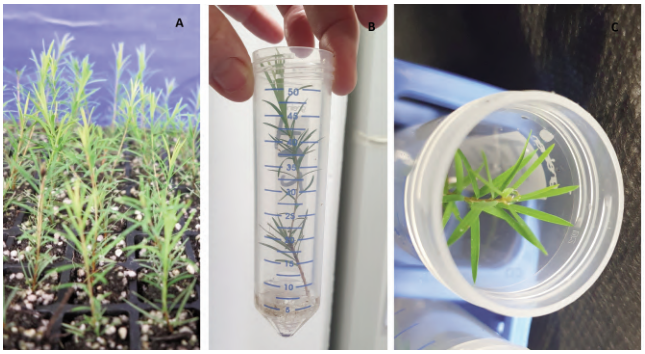

Fig. 1 - Preparing rooted cuttings of tea tree *M. alternifolia* for the GR24 experiment (left); A rooted cutting five months after setting and prior to decapitation (middle); Rooted cutting (control intact) in 50 mL clear plastic tube at the beginning of the experiment, and (right) a droplet of IAA applied on top of decapitated stem stump in GR24 and IAA.

hormone combination treatment (GR24 and IAA), a 0.5mL droplet of IAA was placed on top of the decapitated stem to simulate the presence of the shoot apex and natural auxin production (Cline, 1996) (Fig. 1 right). All plants were given 1 mL of Yoshida nutrient solution (Yoshida *et al.,* 1976) 2 days before treatment solutions, in order to promote growth.

Data collection and analysis

The number of visible axillary buds and axillary shoot length for the 10 uppermost nodes (nodes 1 to 10, with 1 being furthest from the roots) on each plant was recorded at the same time on day 2, 5, 7, 9, 12, 14, 16, 19, and 21. Shoot length was determined to the nearest mm with digital callipers. One-way analysis of variance (ANOVA) was used to test for treatment effects on the number of axillary buds and shoot growth. Where treatment effects were significant ($p<0.05$), a Duncan's multiple range test was performed on treatment means. All statistical analyses were performed using Genstat, Release 19.1 (VSN International, 2018).

3. Results

Effects of GR24 on axillary bud growth in tea tree
Rates of bud release over a 21 day time course experiment

Treatment effect was significant at each of the 9 days counts were undertaken ($p<0.05$) (Fig. 2). No buds were detected on control intact plants until Day 9, and only 40% of control intact plants had one or more detectable buds by 21 days post-treatment (Fig. 2). The rate of bud release on control intact plants was due to normal hormonal regulation in rooted cuttings of tea tree, and it established a baseline for the slowest and least degree of axillary bud development in this study (Fig. 2). In contrast, control decapitated plants had the highest rate of bud development of any of the six treatments as bud release was not mitigated by normal endogenous regulation or due to the presence of exogenous hormonal treatment (Fig. 2). The axillary buds on control decapitated plants began to initiate 2 days after decapitation and continued to develop for the duration of the experiment, with significantly more buds detected at each recording date than any other treatment (Fig. 2). Where exogenous hormones were applied (either

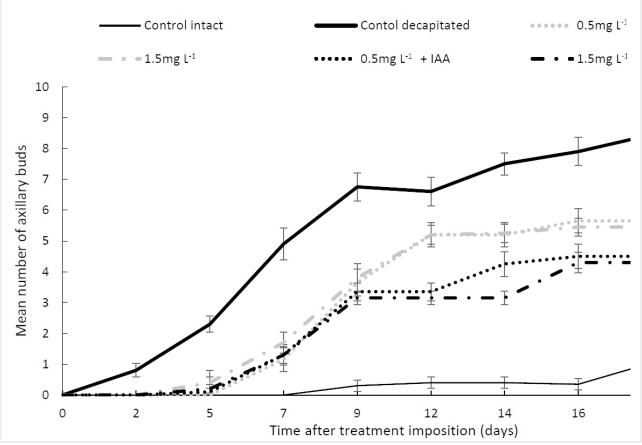

Fig. 2 - Mean number of detectable axillary buds over a 21-day time course for tea tree *M. alternifolia* propagules subjected to GR24 or GR24 plus IAA compared to control intact and decapitated plants. Error bars represent SE of means.

GR24, or GR24 + IAA) the bud numbers were intermediate to the control intact and control decapitated extremes (Fig. 2). All four hormone treatments tended to track together until Day 9, but thereafter, the additional action of auxin apparently moderated the detectable number of buds further (Fig. 2). There was no difference in the number of detectable buds between the low and high GR24 treatment, regardless of IAA presence (Fig. 2).

At the end of the 21-day time course experiment, the control intact plants had significantly less buds (mean + SE 1.1 ± 0.4), and control decapitated plants had a significantly more buds (mean + SE 8.9 ± 0.6), than all other treatments (Fig. 2). While the rate of GR24 (high versus low) had no effect, the addition of

auxin with GR24 resulted in significantly fewer buds relative to GR24 at either rate alone (Fig. 2).

Size and patterns of bud development along the stem

When considering all assessed axillary buds (pooled data from all 10 nodes), as measured on Day 21, hormone treatments of GR24, or GR24 + IAA, significantly reduced the bud size compared to control decapitated plants ($p<0.05$) (Table 1). The average bud length for hormone treatments ranged between 2.5 ± 0.5 mm and 3.3 ± 0.9 mm and was significantly less than the average for control decapitated plants (4.2 ± 0.6 mm), and significantly higher than for control intact plants (0.03 ± 0.08 mm) (Table 1).

Assessment of bud development node by node revealed further differences in developmental patterns. On control intact plants, buds on the uppermost nodes (nodes 1 to 3), if present, were not of a measurable size. In control decapitated plants, buds were longest in the upper most few nodes, and gradually reduced in size to the lowest measured node (node 10) (Table 1). In hormone treated plants, the longest buds occurred at the uppermost nodes (node 2 was generally longer than node 1), and again there was a progressive decline in size moving down the stem (Table 1). Bud length was not typically shorter in hormone treated plants relative to control decapitated plants at the uppermost nodes, but below node 5, hormone treated plants tended to have significantly shorter buds (Table 1). A representative example of the budding response showing bud position and length is presented in figure 3.

4. Discussion and Conclusions

Strigolactone suppressed axillary bud growth in tea tree

Relative to control decapitated plants without GR24 application, application of a synthetic strigolactone analogue, GR24, at rates of 0.5 mg L^{-1} or 1.5 mg L^{-1}, suppressed axillary bud number and expansion in decapitated rooted cuttings of tea tree. This response was consistent with its effects on other species such as calla lily (*Zantedeschia* L. sp.) (Manandhar, 2016) where synthetic SL GR24 was typically applied at concentrations between 0.3 mg L^{-1} (1 µM) and 3 mg L^{-1} (10 µM) (Umehara *et al*., 2008; Manandhar, 2016). Where larger plants at later stages of ontogenetic development (i.e. reproductive maturity) were studied, such as the study of *Chrysanthemum morifolium* Ramat, rates as high as 15 mg L^{-1} (50 µM) were required to inhibit bud growth (Dierck *et al.*, 2016). A moderate response was evident in our tea tree plants at dosages towards the lower end of those used previously with herbaceous species. Both tested rates gave a similar response and, a synergistic effect between SL and auxin was evident, so that bud outgrowth was significantly reduced relative to the application of SL alone. A further factor that may have contributed to the efficacy of the treatments in our study, may have been the retention of the root system. SL is synthesised both in the roots and the shoots and is transported acropetally to suppress bud outgrowth (Domagalska and Leyser, 2011). In most other studies, stem sections with roots

Table 1 - Mean length of the axillary buds from node 1 to node 10 measured at day 21. Treatment effect was tested on an individual node basis

	Control intact	Low GR24 (0.5 mg L^{-1})	High GR24 (1.5 mg L^{-1})	Low GR24+IAA (0.5 mg L^{-1})	High GR24+IAA (1.5 mg L^{-1})	Control decapitated
Average growth (mm)	0.03 ± 0.08 a	2.5 ± 0.5 b	3.3 ± 0.9 b	2.7 ± 0.6 b	2.7 ± 0.6 b	4.2 ± 0.6 c
Node 1 (uppermost)	0 a	7.1 ± 1.1 b	9.8 ± 1.2 bc	9.2 ± 1.5 bc	9.4 ± 1.1 bc	12 ± 1.3 c
Node 2	0 a	8.7 ± 1.1 b	10.5 ± 1.1 bc	9.4 ± 1.3 bc	10.1 ± 0.9 bc	12 ± 0.8 c
Node 3	0 a	5.4 ± 0.9 bc	7.4 ± 1.0 c	4.2 ± 0.9 b	4.5 ± 1.1 b	7.2 ± 0.8 c
Node 4	0.03 ± 0.03 a	2.1 ± 0.9 b	3.2 ± 0.7 bc	2.7 ± 0.9 bc	1.6 ± 0.5 ab	4.6 ± 0.9 c
Node 5	0.1 ± 0.1 a	0.8 ± 0.3 a	1.3 ± 0.4 a	0.8 ± 0.3 a	1.2 ± 0.4 a	2.8 ± 0.7 b
Node 6	0.1 ± 0.1 a	0.2 ±0.05 a	0.7 ± 0.3 a	0.1 ± 0.1 a	0.4 ± 0. 2 a	1.4 ± 0.5 b
Node 7	0.1 ± 0.1 ab	0.4 ± 0.3 bc	0.1 ± 01 ab	0.1 ± 0.04 ab	0 a	0.7 ± 0.2 c
Node 8	0.03 ± 0.03 a	0.6 ± 0.4 b	0.1 ± 0.3 a	0.03 ± 0.03 a	0 a	0.5 ± 0.1 b
Node 9	0 a	0.1 ± 0.1 a	0 a	0.03 ± 0.03 a	0 a	0.2 ± 0.1 b
Node 10 (lower most)	0.1 ± 0.1 a	0.03 ± 0.03 a	0 a	0 a	0 a	0.2 ± 0.1 b

Treatment means followed by the same letter within the same row are not significantly different at the 95% confidence level (- denotes no bud present).

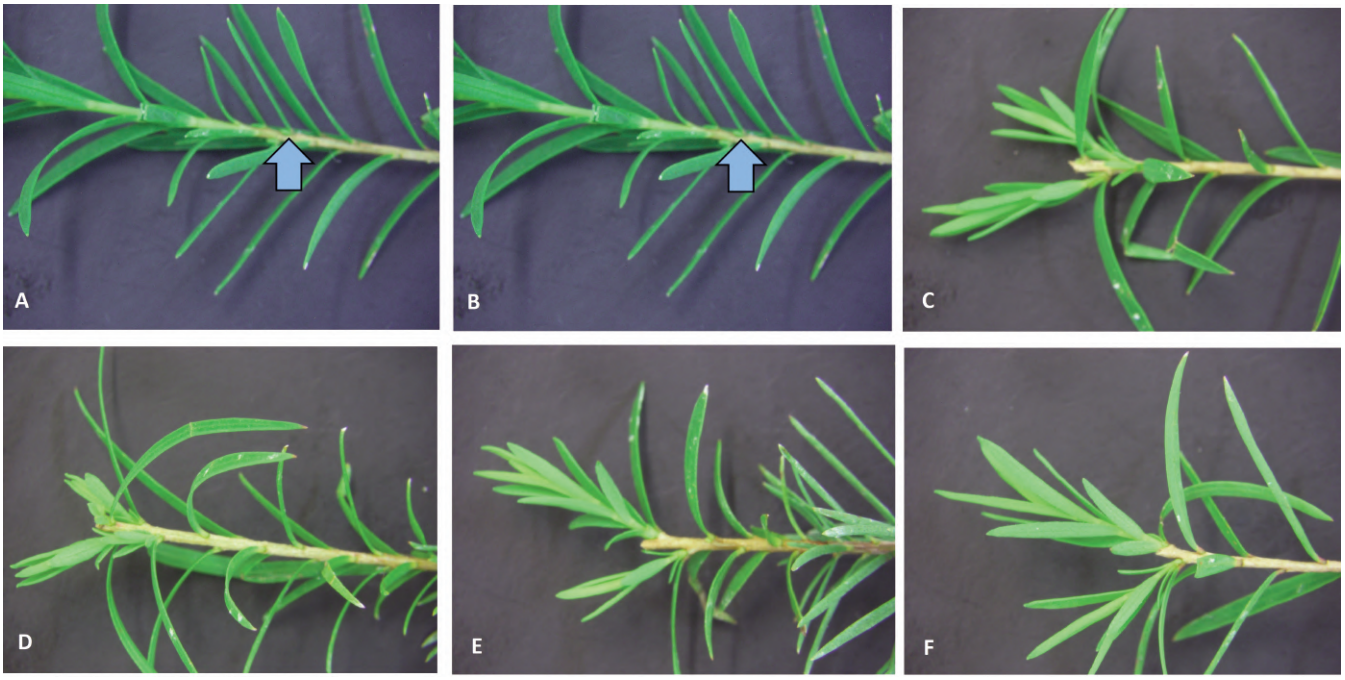

Fig. 3 - A representative *M. alternifolia* propagule from each treatment showing the pattern and size of axillary buds 21 days after treatment. Figure 3a, is a control intact propagule, buds were not visible without magnification at the upper nodes. Buds were visible from node 4 or lower but did not develop (arrow). Figure 3b, is a control decapitated propagule, with axillary buds at upper nodes developed into side shoots (arrow). Figures 3c to 3f show a propagule from each of the GR24 and GR24 plus IAA treatments, buds and developing side shoots visible only on the upper most nodes with generally less shoot growth compared to the control decapitated propagule in figure 3b.

removed were used for experimentation, and SL was supplied to the basal stem stump. Therefore endogenous SL may have reinforced any effect due to exogenous GR24 in our case, potentially inducing a stronger (bud outgrowth inhibition) response in the lower range of concentrations of GR24 compared to studies on other plants.

Axillary bud outgrowth further inhibited with the addition of an auxin supply

When GR24 was applied in conjunction with auxin, suppression of axillary bud outgrowth was enhanced relative to exogenous GR24 alone, as reported in other species (Crawford *et al.,* 2010; Liang *et al.*, 2010; Ward *et al.,* 2013). Liang *et al.* (2010) reported total inhibition of bud outgrowth when 1.5 mg L^{-1} (5 µM) of GR24 and NAA were applied to chrysanthemum (*Dendranthema grandiflorum*) stems. Although both GR24 and GR24 plus IAA (at the low and high rate) treatments inhibited bud outbreak in tea tree plants for 5 days, when the buds began to grow and elongate, the growth rate of the buds was comparable to the control decapitated plants, at least for the upper four nodes for most plants (Table 2). These observations provide further support for the influence of auxin in the vascular stream of the main stem on bud release and the influence of auxin transport on SL inhibition of axillary buds. Ultimately, our findings are consistent with both the canalisation (Brewer *et al.*, 2009; Ljung *et al.*, 2001) and second messenger (Domagalska and Leyser, 2011) models developed to explain the physiological regulation of shoot architecture involving the interaction plant hormones including auxin, CK and SL (Gomez-Roldan *et al*., 2008; Domagalska and Leyser, 2011).

Competition between buds

The role of the GR24 in reducing bud release and growth needs to be considered along with the inhibitory effect active buds have on buds above or below it and those opposite to it (Thimann and Skoog, 1934). Lateral buds do not produce auxin while they remain dormant but produce considerable quantities when actively growing (Balla *et al.*, 2011). Auxin synthesised in an active bud is transported into

the main stem, and this auxin saturation prevents auxin movement out of axillary buds further down the stem, inhibiting bud growth (Leyser, 2005; Balla *et al.*, 2011). Tea tree plants treated with GR24 or GR24 + IAA, buds tended to form on the upper nodes (node 1 to node 10), whereas buds formed down to node 14 on control decapitated plants (Table 1; data >node 10 not shown). The rapidly growing buds on the upper nodes of control decapitated plants did not appear to inhibit bud release on the nodes below them, but they did affect subsequent bud elongation, consistent with a requirement for SL for bud inhibition (Beveridge, 2000). The mean length of the buds on the control decapitated plants decreased significantly for the majority of nodes, basipetally ($p < 0.05$) (Table 1). For plants given the higher rates of GR24 (with and without IAA), active buds may have contributed to the suppression of bud outbreak on the lower nodes, as no bud outbreak occurred past node 6 and 8, for GR24 and GR24 +IAA respectively (Table 1).

Implications for use of SL analogues as agents for manipulating tea tree nursery stock

This study demonstrated the potential of synthetic SL GR24 at relatively low concentrations of 0.5 mg L^{-1} and 1.5 mg L^{-1} to suppress bud outgrowth in tea tree. This was encouraging for a potential application of SL in an early intervention response to mitigate undesirable side branching of plants where shoot tips are inadvertently lost due to pest, heat or desiccation damage, or where tips are removed during propagation (i.e. use of nodal cuttings). None of the artificial treatments tested were as effective as the control intact treatment where apical dominance suppresses bud outgrowth, but a moderate response at relatively low dosages, suggests there is room to explore whether higher dosages can elicit stronger suppression of side branching. For example, Liang *et al.*, (2010) found higher concentrations of exogenous SL or SL with auxin suppressed bud outgrowth to the same degree as endogenous regulation induced by an intact apical buds in chrysanthemum.

The response at lower dosages in this first investigation was also encouraging because woody perennial species do not appear to be recalcitrant to the influence of hormones, at least for our relatively small (less than 2 grams fwt of biomass) test plants, with root systems, and with low tissue specialisation (i.e. stems had low degrees of lignification and had not formed papery bark). Further work will be required, however, to establish the efficacy of higher dosages to elicit stronger suppression responses in nursery propagules, the longevity of the effect of exogenous SL on the form of the propagule (i.e. does it persist to suppress side shoots on larger plants ready for release from the nursery?), and whether there is any unfavourable longer term impact of plant growth in the field.

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References

ANPSA 2012 - Melaleuca, *Propagation*. - Australian Native Plant Society Australia. http://www.anpsa.org.au/melaleu2.html.

BALLA J., KALOUSEK P., REINOHL V., FRIML J., PROCHAZKA S. 2011 - *Competitive canalization of PIN-dependent auxin flow from axillary bud controls pea bud outgrowth*. - The Plant J., 65: 571-577.

BEVERIDGE C.A., 2000 - *Long-distance signalling and mutational analysis of branching in pea.* - J. Plant Growth Regul., 32: 193-203.

BREWER P.B., DUN E.A., FERGUSON B.J., RAMEAU C., BEVERIDGE C.A., 2009 - *Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and* Arabidopsis. - Plant Physiol., 150: 482-493.

CARSON C.F., HAMMER K.A., RILEY T.V., 2006 - *Melaleuca* alternifolia *(Tea tree) oil: a review of antimicrobial and other medicinal properties.* - Amer. Soc. Microbio., 19(1): 50-62.

CLINE M.G., 1996 - *Exogenous auxin effects on lateral bud outgrowth in decapitated shoots*. - Ann. Bot., 78(2): 255-266.

CRAWFORD S., SHINOHARA N., SIEBERER T., WILLIAMSON L., GEORGE G., HEPWORTH J., MULLER D., DOMAGALSKA M., LEYSER O., 2010 - *Strigolactones enhance competition between shoot branches by dampening auxin transport.* - Develop., 137: 2905-2913.

DIERCK R., DE KEYSER E., DE RIEK J., DHOOGGHE E., VAN HUYLENBROECK J., PRISEN E., VAN DER STRAETEN D., 2016 - *Change in auxin and cytokinin levels coincides with altered expression of branching genes during axillary bud outgrowth in Chrysanthemum.* PLoS ONE 11:e0161732.

DOMAGALSKA M., LEYSER O., 2011 - *Signal integration in the control of shoot branching*. - Nat. Rev. Mol. Cel. Biol., 12: 211-221.

DORAN J.C., BAKER G.R., MURTAG G.J., SOUTHWELL I.A., 1997 - *Improving tea tree yield and quality through breeding and selection*. - Report for the RIDC, Canberra, pp. 1-50.

DUN E.A., DE SAINT G.A., RAMEAU C., BEVERIDGE C.A., 2012 - *Antagonistic action of strigolactone and cytokinin in bud outgrowth control.* - Plant Physiol., 158(1): 487-498.

DUN E.A., DE SAINT G.A., RAMEAU C., BEVERIDGE C.A., 2013 - *Dynamics of strigolactone function and shoot branching responses in* Pisum sativum. - Mol. Plants, 6: 128-140.

FERGUSON B.J., BEVERIDGE C.A., 2009 - *Roles of auxin, cytokinin, and strigolactone in regulating shoot branching*. - Plant Physiol., 149: 1929-1944.

FERRERO M., PAGLIARANI C., NOVÁK O., FERRANDINO A., CARDINALE F., VISENTIN I., 2018 - *Exogenous strigolactone interacts with abscisic acid-mediated accumulation of anthocyanins in grapevine berries*. - J. Exp. Bot., 69: 2391-2401.

GOMEZ-ROLDAN V., FERMAS S., BREWER P.B., PUECH-PAGES V., DUN E.A., PILLOT J.P., LETISSE F., MATUSOVA R., DANOUN S., PORTAIS J.C., BOUWMEESTER H., BECARD G., BEVERIDGE C.A., RAMEAU C., ROCHANGE S.F., 2008 - *Strigolactone inhibition of shoot branching*. - Nature, 455: 189-194.

LEYSER O., 2005 - *The fall and rise of apical dominance*. - Curr. Opin. Genet. Dev., 15(4): 468-471.

LEYSER O., 2009 - *The control of shoot branching: an example of plant information processing.* - Plant Cell Environ., 32: 694-703.

LIANG J., ZHAO L., CHALLIS R., LEYSER O., 2010 - *Strigolactone regulation of shoot branching in chrysanthemum* (Dendranthema grandiflorum). - J. Exper. Bot., 61(11): 3069-3078.

LJUNG K., RISHIKESH P., SANDBERG G., 2001 - *Sites and homeostatic control of auxin biosynthesis in* Arabidopsis *during vegetative growth*. - Plant J., 28(4): 465-474.

LOWE G.E., SHEPHERD M., ROSE T., RAYMOND C., 2019 - *Mini cuttings improve the shoot and root architecture of tea tree,* Melaleuca alternifolia *(Maiden & Betche) Cheel*. - Propag. Ornam. Plants, 19(4): 95-105.

MANANDHAR S., 2016 - *Strigolactones and hormonal interaction in control of branching in* Zantedeschi*a and other horticultural species*. - PhD Thesis, Massey University, Palmerston North, New Zealand, pp. 228.

MULLER D., LEYSER O., 2011 - *Auxin, cytokinin and the control of shoot branching*. - Ann. Bot., 107: 1203-1212.

SHEPHERD M., ROSE T., RAYMOND C., 2013 - *Rejuvenation of mature native tea tree* (Melaleuca alternifolia *(Maiden & Betche) Cheel. for vegetative propagation*. - Propag. Ornam. Plants, 13: 103-111.

THIMANN K.V., SKOOG F., 1934 - *On the inhibition of bud development and other functions of growth substance in* Vicia faba*.* - Proc. Royal. Soc. B., 114: 317-339.

UMEHARA M., HANADA A., YOSHIDA S., AKIYAMA K., ARITE T., TAKEDA-KAMIYA N., MAGOME H., KAMIYA Y., SHIRASU K., YONEYAMA K., KYOZUKA J., YAMAGUCHI S., 2008 - *Inhibition of shoot branching by new terpenoid plant hormones*. - Nature, 455: 195-200.

VSN International, 2018 - Genstat for Windows 19th Edition. - VSN Int. l, Hemel Hempstead, UK.

VURRO M., PRANDI C., BAROCCIO F., 2016 - *Strigolactones: how far is their commercial use for agricultural purposes?* - Pest Manag., 72: 2026-2034.

WARDS P., SALMON J., HANLEY S.J., KARP A., LEYSER O., 2013 - *Using* Arabidopsis *to study shoot branching in biomass willow*. - Plant Physiol., 162: 800-811.

YAISH M.W.F., GUEVARA D.R., EL-KEREAMY A., ROTHTSEIN S.J., 2010 - *Axillary shoot branching in* plants, pp. 37-52. - In: PUA E.C., and M.R. DAVEY (eds.) *Plant developmental biology. Biotechnological perspectives*. Springer-Verlag Berlin, Heidelberg, Germany, pp. 485.

YOSHIDA S.I., FORNO D.A., COCK J.H., GOMEZ K.A., 1976 - *Laboratory manual for physiological studies of rice*. - Inter. Rice Research Institute, Manila, Philippines.