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Pruning date and hydrogen cyanamide effects on growth and yield of grapevine var. Cabernet Sauvignon

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Abstract: Harvesting the grapes before the monsoon season is crucial to ensure the quality of berry and bunches. This study aims to identify the optimal window for pruning and hydrogen cyanamide (HC) application to prepone the berry harvesting. The experiment was conducted in randomized complete block design with five treatments and four replications. Treatments were five different pruning dates in 2021: Jan. 17, Jan. 24, Jan. 31, Feb. 7 and Feb. 14, followed by 5% HC application one week after pruning. Annual growth stages of grapevine were recorded by using modified E-L growth stage; reproductive attributes recorded during flowering; and vine yield and berry quality attributes recorded at harvesting. The earlier pruning resulted earlier budburst compared to late pruning. Vines prunefed after Feb. 7 had <50% budburst, while vines pruned before Feb. 7 reached 50% budburst, exhibiting no differences in number of days needed to achieve it. Jan. 17 pruning had the highest budburst (%) and bud fruitfulness (%), where pruning on Feb. 7 had lower values. Average bunch weight did not differ while berry quality attributes differed between treatments for the same day harvest. The negative responses in late pruned and HC treated vines, potentially attributed to the phytotoxic effect of HC on tender buds near the natural time of dormancy break. The early pruning and subsequent application of HC triggered earlier budburst, and advances flowering and harvesting of berries in grapevine. This research demonstrated a potential techniques for advancing harvesting time (2-3 weeks) in grapevine.

1. Introduction

Grape (*Vitis vinifera* L.) is a non-climacteric berry fruit of the deciduous woody vines belonging to the family Vitaceae, indigenous to Eurasia (This *et al.*, 2006). Grapes can be consumed in fresh or processed products like juice, wine, vinegar, jelly, jam, grape seed oil, and raisins. Cabernet Sauvignon is a widely cultivated grape variety for wine due to its small berries with a higher concentration of tannin and coloring pigment (Robinson *et al.*, 2012). Grape cultivation in Nepal was started about 70

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years ago but there was no significant expansion of viticulture farms (Dahal *et al.*, 2017). Currently, few commercial vineyards are producing mainly wine grapes while table grapes are in small quantities. The estimated production of Nepal was 76 t from 20 ha area with a productivity of 8.5 t ha^{-1} (Atreya *et al.*, 2015) but the demand of grape has been increasing manifolds in recent years (Acharya *et al.*, 2023). It shows there is a huge demand for grapes along with bottlenecks of Nepalese viticulture. The main challenge is harvesting time coincides with the rainy season causing a high risk of fungal diseases and insect infestation during ripening resulting in inferior quality berries and bunch (Shrestha, 1998). Such problems are also common in humid subtropical areas in other region too.

Grapevine dormant buds starts the resumption of annual growth cycle after winter in most of the subtropical and temperate climates. Chilling requirement and breaking dormancy are crucial affecting temperate fruit trees when grown in tropical climates (Botelho and Müller, 2007). Various chemicals, such as mineral oil (Black, 1936), Dinitro-ortho-cresol (Erez and Sur, 1981), Thiourea (Blommaert, 1965), Garlic extract (Botelho and Müller, 2007), and HC have been used to break dormancy in grapevine. Among these chemicals, HC has great efficiency in bud breaking (Nir and Levee, 1993) as well as enhances uniform and rapid bud breaking (McColl, 1986; Halaly *et al.*, 2008). However, the effect of HC depends upon the time and concentration used. Hydrogen cyanamide breaks endo-dormancy by respiratory disturbance, hormonal signaling, and oxidative stress (Liang *et al.,* 2019). This research was purposed to advance the natural budburst period, which might lead to bunches being harvested before monsoon. To harvest the crop before monsoon, breaking of bud dormancy earlier than natural time is required. In the warmer climates, pruning followed by HC are employed to induce budburst. Aiming to prepone natural budburst and to allow the berry harvesting before the heavy rainfall or monsoon.

2. Materials and Methods

The experiment was conducted from January 17 to July 04, 2021, at the commercial vineyard (27°44’ N, 85°6’ E) in Dhading, on a south-facing plot with a gentle slope at an altitude of 800 meters above mean sea level. The grapevine cv. Cabernet Sauvignon (6 years old) grafted on 5C rootstock, was selected for the research. Five pruning dates followed by HC application treatments was arranged on a Randomized Complete Block Design with five replications considering a vine as replication (Table 1).

Table 1 - Description of treatment details and coding of treatment practiced in a commercial vineyard, Dhading, Nepal, 2021

Treatments	Pruning date	5% HC application date	Treatment code
T1	January 17	January 24	J17J24
T2	January 24	January 31	J24J31
T3	January 31	February 07	J31F07
T4	February 07	February 14	F07F14
T5	February 14	February 21	F14F21

To differentiate treatments, vines within replication were tied with different colored ribbons. One-year-old vines were spur-pruned, leaving three basal buds per spur. Ten spurs from each vine were selected and tagged with different colored threads. Buds in spur were marked as 1, 2, and 3 from the basal to distal. Hence, 30 buds were marked per vine. Phenological observations using the modified Eichhorn and Lorenz (E-L) grapevine growth stages scale began on February 16, 2021, and carried out in every four days interval until April 26, 2021. The growth stages, reproductive attributes during flowering, vine yield, and berry quality attributes were recorded at harvest. Development parameters such as budburst, fruitfulness in total buds, and fruitfulness in burst buds were calculated using the following formulas:

Budburst (%) = Number of burst buds/Total buds x 100

Observed fruitfulness (%) = Number of buds with inflorescence(s)/ Total buds x 100

Fruitfulness in burst buds (%) = Number of buds with inflorescence(s) / Total burst buds x 100

All bunches of each treatment were harvested on the same day, July 4, 2021 to ensure that the minimum standard quality berries and bunches harvested before the monsoon arrives. Quantitative attributes were measured from randomly selected 10 bunches. The qualitative attributes (Total soluble solid and Total titratable acidity) were assessed by randomly selected 10 berries from each selected bunch (Dahal *et al.*, 2019). Data recorded from the field were entered, tabulated and analyzed using MS Excel 12 and GENESTAT version 18.1.

3. Results

Phenological observations

Annual growth stages of grapevine. Considerable variation in average E-L growth stages among different treatments were observed throughout the experimental period as shown in Table 2. Lower values were recorded in later treated vines (F07F14 and F14F21) while early treated (J17J24 and J24J31) vines had higher values of E-L stages. At the last date of observation (26th April), the average E-L stage of J17J24 and F07F14 were 19.69±6.27 and 3.21±2.98, respectively.

Number of days to budburst. The number of days to first budburst differed significantly between treatments while treatments did not significantly differ in days to 50% budburst (Table 3). Budburst was earlier

Table 3 - Effect of pruning date followed by HC application on number of days to 1st and 50% budburst, Dhading, Nepal, 2021

Treatments	Days to the first budburst	Days to 50% budburst
J17J24	0.8 ±0.8 a	16.8 ±2.8
J24J31	8.0 ±1.26 ab	17.2 ±0.8
J31F07	10.4 ±1.6 b	19.0±1.03
F07F14	26.4 ±3.7 c	NA
F14F21	24.8 ±4.08 c	NA
Grand mean	14.1	17.67
LSD	7.93	NA
F-probability	<0.001	NA
CV%	42.0	19.1

Mean with the same letter(s) within the column do not differ significantly by DMRT at 5%. Values are μ±SE where μ = Mean and SE = Standard error. LSD=Least Significance Difference. CV = Coefficient of variance. NA= Not applicable.

Table 2 - Average E-L stage of grapevine buds in different dates of pruning and HC application, Dhading, Nepal, 2021

Observation day	Average E-L growth stage in treatments					Statistical analysis	
	J17J24	J24J31	J31F07	F07F14	F14F21	Grand mean	LSD (α =5)
D0	1.39±0.38 c	1.07±0.11 b	1.03±0.07 b	1.01±0.04 a	1.01±0.04 a	1.10	0.105 **
D4	1.73±0.52 d	1.33±0.24 c	1.16±0.16 b	1.01±0.04 a	1.01±0.04 a	1.25	0.101 **
D8	2.23±0.8 d	1.71±0.45 c	1.31±0.26 b	1.02±0.06 a	1.01±0.05 a	1.47	0.279 **
D12	3.26±1.17 d	2.39±0.76 c	1.73±0.38 b	1.05±0.10 a	1.03±0.07 a	1.89	0.514 **
D16	4.97±1.69 d	3.76±1.4 c	2.71±0.88 b	1.1±0.18 a	1.09±0.15 a	2.72	0.836 **
D20	6.11±1.92 c	5.08±1.84 c	4.14±1.55 b	1.17±0.33 a	1.17±0.24 a	3.53	1.044 **
D24	7.74±2.34 c	6.57±2.29 bc	5.65±2.11 b	1.27±0.46 a	1.43±0.45 a	4.53	1.392 **
D28	8.7±2.55 c	7.25±2.48 bc	6.08±2.24 b	1.39±0.61 a	1.78±0.73 a	5.07	1.527 **
D32	9.35±2.76 c	8.05±2.73 bc	6.86±2.52 b	1.51±0.77 a	2.14±0.95 a	5.61	1.735 **
D36	10.85±4.75 c	8.9±3.02 b	8.11±2.91 b	1.71±1.04 a	2.85±1.39 a	6.48	1.895 **
D40	11.16±3.26 c	9.93±3.38 bc	8.48±3.02 b	1.91±1.29 a	3.36±1.71 a	6.97	2.161 **
D46	12.43±3.63 c	11.1±3.78 bc	9.69±3.46 b	2.12±1.54 a	4.25±2.27 a	7.92	2.510 **
D49	13.79±4.04 c	11.98±4.13 bc	10.58±3.93 b	2.31±1.8 a	4.87±2.62a	8.13	3.017 **
D51	15.1±4.56 b	12.76±4.53 b	12.57±4.52 b	2.50±2.03 a	5.49±3.02 a	9.70	3.525 **
D55	16.09±5 b	13.82±5.02 b	13.61±5.02 b	2.69±2.28 a	6.21±3.49 a	10.48	3.725 **
D59	16.9±5.28 b	14.72±5.32 b	14.63±5.35 b	2.87±2.5 a	6.97±3.97 a	11.21	4.140 **
D65	18.33±5.74 b	16.26±5.92 b	16.00±5.86 b	3.05±2.75 a	7.83±4.52 a	12.25	5.081 **
D69	19.69±6.27 b	17.65±6.46 b	17.53±6.46 b	3.21±2.98 a	8.62±5.06 a	13.29	5.679 **

D= Date on phenological observation was done. D0 = 16th Feb.; D4 = 20th Feb.; D8 = 24th Feb.; D12 =28th Feb.; D16 =4th Mar.; D20 = 8th Mar.; D24 = 12th Mar.; D28 = 16th Mar.; Day32= 20th Mar.; D36 =24th Mar.; D40 = 28th Mar.; D46 = 3rd Apr.; D49 =6th Apr.; D51= 8th Apr.; D55 = 12th Apr.; D59 = 16th Apr.; D65 = 22nd Apr.; D69= 26th Apr.; Values are μ ± SE where μ = Mean stage; LSD = Least Significance Difference; ** highly significant at α =5%; NS = Not significant.

in the early pruned vine as compared to late pruned grapevines. Early pruned (Jan. 17) grapevine burst their first bud in 0.8±0.8 days while late pruned vines (Feb. 7) took 26.4±3.7 days after HC application.

Growth and development observations

Budburst percentage. The overall budburst was less than 50% for all treatments. Significant differences between treatments were found in different observation dates after treatment application. Early pruned and HC treated vines (J17J24, J24J31, and J31F07) were not only early in budburst, but they also had higher budburst (%) compared to late pruned and HC treated vines. Late pruned and HC treated vines had lower budburst (%) even around 10% as shown in figure 1. HC application close to natural budburst time damages the buds and buds did not sprout (<4 E-L growth stage), hence late pruned and HC treated (F07F14 and F14F21) vines did not reach the 50% budburst.

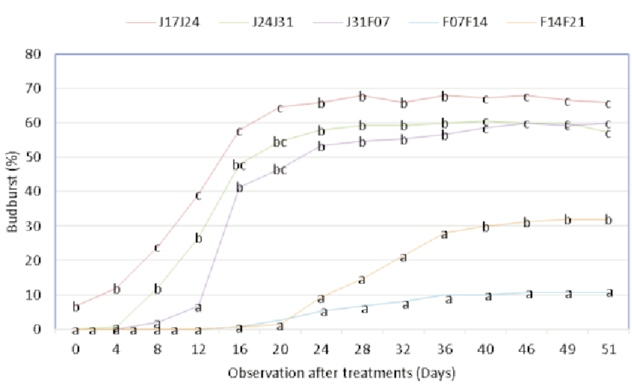

Fig. 1 - Effect of different date of pruning followed by HC application on budburst (%). Means are separated with different letter(s) for the respective day of observation using Duncan's multiple range test at 5%.

Observed fruitfulness in total buds. The overall flowering of all treatments was less than 40%, however, significant difference was observed between treatments on different dates of observations (Fig. 2). Flowering (%) variation among treatments follows a similar trend to that of budburst percentage as shown in figure 1 and figure 2. Late pruned and HC treated vines had lower flowering (%) and delayed in flowering. Vine took 40-49 days to flower after budburst. Late pruned and HC treated vines started to flower after 49 days while earlier treated vines started flowering after 40 days.

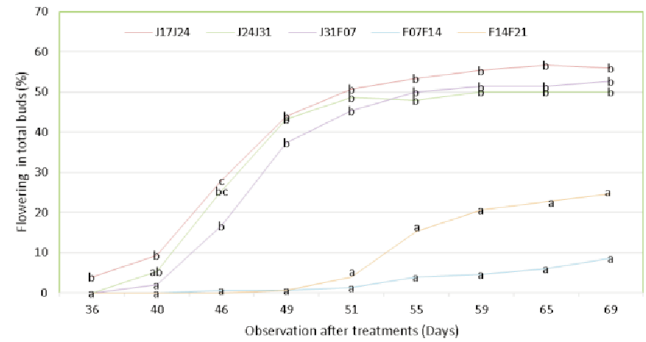

Fig. 2 - Effect of different timing of pruning followed by HC application on observed fruitfulness. Means are separated with different letter(s) for the respective day of observation using Duncan's multiple range test at 5%.

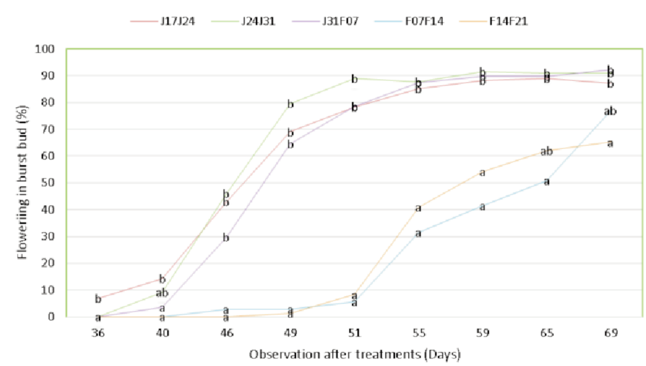

Fig. 3 - Effect of different timing of pruning followed by HC application on observed fruitfulness. Means are separated with different letter(s) for the respective day of observation using Duncan's multiple range test at 5%.

Fruitfulness in burst buds. Flowering percentage in burst bud was insignificant between treatments from the last date of bud observation (Fig. 3). On April 26, the flowering (%) observed in the burst bud was 82.6% with the death of average 10% buds and the remaining bud did not reach the flowering stage. Treatments J17J24, J24J31, J31F07, F07F14, and F14F21 had bud death percentage as 14.28%, 13.41%, 6.98%, 6.25%, and 4% of total burst buds, respectively.

Yield attributes. The TSS and TA of berries were significantly different between treatments (Table 4). Early pruned and HC treated vines produced berries with higher TSS and lower TTA. TSS and TTA of J17J24, J24J31, J31F07, and F07F14 were statistically similar to each other while F14F21 had a higher TTA and lower TSS value. The average bunch weight was similar for all treatments (Table 4).

Table 4 - Effect of pruning date followed by HC application on yield attributes of grapevine, Dhading, Nepal, 2021

Treatments	Total soluble solid (˚B)	TTA (g/L tartaric acid)	Average bunch weight
J17J24	18.96±0.55 b	9.93±0.81 a	69.56±14.31
J24J31	19.26±0.59 b	9.93±0.32 a	62.86±8.81
J31F07	18.84±0.89 b	10.27±0.94 a	70.06±6.98
F07F14	17.98±0.43 b	10.87±0.57 a	74.92±12.04
F14F21	16.08±0.71 a	12.72±0.3 b	70.05±6.70
Grand mean	18.22	10.75	69.5
LSD (5% level)	1,829	1,537	na
F-probability	0.013 **	0.00 **	0.96 ns
CV (%)	7.5	10.7	35.50

Mean with the same letter(s) within the column do not differ significantly by DMRT at 5%. Values are µ±SE where µ = Mean and SE = Standard error. LSD=Least Significance Difference. CV = Coefficient of variance. NA= Not applicable.

4. Discussion and Conclusions

This study showed the growth and phenological stages were significantly influenced by the date of pruning followed by 5% HC application in grapevine cv. Cabernet Sauvignon. Budburst depends on warm or forcing conditions in some cultivars after endo-dormancy (Keller and Tarara, 2010). The general threshold temperature for shoot development is 10°C. To some extent, the budburst date corresponds to the cumulated temperatures above this threshold (Lebon *et al.*, 2004). Martin and Dunn (2000) found that HC did not significantly affect the times of the onset of budburst, 60% budburst, anthesis or veraison, or fruit maturity at harvest, but interacted significantly with later pruning to delay fruit maturity. Martin and Dunn (2000) reported that six-week differences in pruning time resulted in 5 days differences in budburst time in the cultivar Cabernet Sauvignon in Melbourne, Australia. HC treatment prepone budburst of vine due to increased accumulation of H_2O_2, soluble sugar/starch ratio, IAA, and cytoplasmic protein-tyrosine kinase concentration with decreased ABA concentration (Liang *et al.*, 2019). Delayed winter pruning postponed 10-11 days for budburst which reported a possible solution to prevent spring freeze damage (Persico *et al.,* 2021).

In late pruned and HC treated vine, dramatic decrease in budburst was observed. Low budburst is potentially linked to phytotoxic effects caused by late application of HC. As the natural budburst time approaches, buds are succulent and vulnerable to the toxic effect of HC. George and Nissen (1988), George *et al.* (1988), and Shulman *et al.* (1983) also reported the drying out of young shoots due to too early or late application of HC. Early burst bud may dry out as succulent and young shoots have to face the frost. In most fruit trees, the biggest effect of HC reported when applied few weeks before the natural budburst (Pontikis, 1989). While the late application (F14F21) may not have an impact because the chemical resistance reduces quickly after being released from endo-dormancy (Snir, 1988; Klinac *et al.*, 1991). Using two different HC application dates (mid-Dec. and mid-Jan.), Or *et al.* (1999) reported that there was no discernible difference in the budburst (%). In both dates, the budburst was found to be 50% after four weeks and an additional 20% within the following two weeks. Cabernet Sauvignon has a shorter duration of budburst to flowering period as compared to Merlot and Cabernet Franc in Bordeaux, France (Leeuwan *et al.*, 2004). Further they reported that the low yield was typically associated with low budburst rates, while HC influence on grapevine yield has been attributed to its impact on budburst. A high level of budburst would result in an increased shoot number and, therefore, a high yield (Or *et al.*, 1999). The phytotoxic effect of HC acting upon naturally burst tender buds has been reported since its early application (Shulman *et al.*, 1983).

Lower budburst and flowering in late pruned and HC treated vines result in lower fruitfulness. Bud fruitfulness depends upon climatic variables mainly sunshine and daily maximum temperature between 82°F and 90°F and water availability (Williams, 2000). Unfavorable climatic conditions hasten diseases and insect infestation such as anthracnose, Downy mildew, mealy bugs, thrips, and leaf hoppers that result in burst bud mortality (Somkuwar *et al.*, 2021).

Vine productivity is a distinguishing feature of a variety that fluctuates depending on several parameters, such as rootstock, and vine management. The significance of pruning in the grapevine is relatively consistent (Rives, 2000). Martin and Dunn (2000) found that the earlier pruned (7 July) vines matured earlier than the later pruned (17 August) vines, and the mean TSS of the berry was 0.91°B lower for the later pruned vines. Dhakal (2021) found insignificant difference between the average bunch weight of vine pruned at different times followed by HC application in Kirtipur, Nepal.

This research demonstrated the advancing budburst date through pruning and HC application ultimately advances the harvesting time. Thus, it can be a potential and viable strategy to address the challenges of monsoon coinciding with harvesting time in grapevine growing areas. This study demonstrated that shoot pruning during second fortnight of January followed by 5% HC application advances 2-3 weeks in harvesting of grapevine without compromising in minimum acceptable berry quality. Particularly, in the subtropical conditions of Nepal, it is recommended to apply HC before Feb. 7th to optimize its effects with 5% HC application.

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