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HYDROGEN CYANAMIDE: A CHEMICAL TO PREPONE NATURAL BUDBURST TIMING OF GRAPEVINE CULTIVARS IN NEPALESE CONTEXT

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ABSTRACT

Grapes primarily belongs to mediterranean climate but overtime due to successful vine management practices, its cultivation has been extended to sub-tropics and tropics. In Nepal, grape cultivation has been started about 8 decades ago, and the demands of this crop has been increasing every year. However, the critical challenge of heavy rainfall coinciding with the berry harvest period has limited the production window, necessitating innovative solutions. This study explores the potential of grape cultivation in Nepal by assessing significance of hydrogen cyanamide (HC) application to ensure early and uniform budburst and therefore fruit maturity before monsoon. Winter pruning and HC application at 2-5% concentration has been found effective in preponing budburst and maturity in some grapes varieties 'Steuben' and 'Cabernet Sauvignon' in Nepal, but the results depend on date and dose of HC application in particular variety and specific growing condition. Application of HC at 2% concentration has resulted in earlier budburst by about 3 weeks in var. Cabernet Sauvignon in Dhading, Nepal. Also, the earlier treatment of HC and pruning in comparison to normal time of pruning (December 15 to January 15) has resulted in earlier and higher budburst and observed fruitfulness in varieties like 'Steuben' and 'Cabernet Sauvignon' in warm temperate belt of Nepal. Thus, the study concludes the prepone of natural budburst is a must need practice for successful viticulture in terai and mid-hills of Nepal. It also emphasizes the need of more research to optimize the application of HC and ensure its efficacy in advancing budburst and maturity specific to variety and locality. Overall, the findings aim to inspire growers, enhance commercial viability, and contribute valuable insights to the viticulture sector in Nepal.

Key words: Budburst, Dormancy, Grapevine, Hydrogen cyanamide, Pre-pone

1. INTRODUCTION

Grape (*Vitis* spp.) cultivation in Nepal is believed to have been started within the Rana regime (>75 years ago). The estimated area under grape cultivation is about 40 ha in 2022 (Acharya, Acharya, Kushwaha, & Dahal, 2023) with estimated total fresh production of about 76 tonnes per annum in Nepal (Atreya, Lamichhane, & Kafle, 2015). Small vineyards on government research stations/farms were established in temperate and warm temperate climates from 1968 AD. However, viticulture has not been much prioritized and commercial grape cultivation is only limited to few farms like Kewalpur Agro Farm Vineyard, Dhading.

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Grapes were primarily grown in mediterranean type of climate (hot and dry summer followed by cool winter) (Arroyo-García et al., 2006). Over time, grape cultivation has been extended over subtropical to tropical climatic regions including China, India, Brazil, Turkey etc (Jones, White, Cooper, & Storchmann, 2005). In mediterranean climates, budburst starts in spring and is followed by a period of 5-6 months to crop harvesting (Mullins, Bouquet, & Williams, 1992), whereas in warmer climates the period to crop harvesting is shorten about three months after budburst (Dahal, Bhattarai, Midmore, Oag, & Walsh, 2017). In the regions with a summer followed by monsoon and a short-mild winter, a single pruning with forcing of budburst and a single harvest is the accepted practice (Lavee, 2000).

In Nepal, monsoon coinciding harvest and associated diseases have been considered the major limitations for successful grape cultivation. Grape technology development projects planted grapevines in eastern terai and then to western terai during 1980's and 1990's but were heavily infected by diseases (Joshi, 1986; Shrestha, 1998). The monsoon coincided with harvesting time reduces the quality of berry and invites the high humidity associated diseases. Thus, the bottleneck for successful viticulture in Nepal is the harvesting time coincides with monsoon period (Atreya et al., 2015). In 2017, Warm Temperate Horticulture Centre, Kirtipur failed to harvest 40% of total bunch harvest due to early rains during in the monsoon. Innovative techniques to shift harvesting time earlier than monsoon has been a common practice but has not yet adopted in Nepalese viticulture (Dahal et al., 2017).

Shifting of natural budburst time 20-30 days earlier than the natural budburst time hastened the crop maturity by at least 20 days that skip the overlapping period of berry maturity with calendar monsoon in the central part of Nepal. The key regulators of bud dormancy are temperature, especially accumulated chilling time, and photoperiod (Carmona, Chaïb, Martínez-Zapater, & Thomas, 2008; Fennell & Hoover, 1991). Due to suboptimal chilling temperature and a brief winter in subtropical climates, the application of a dormancy breaking chemical i.e. Hydrogen cyanamide (H_2CN_2 syn. HC) followed by pruning in winter is vital to induce uniform budburst (Lavee & May, 1997; Lombard, Cook, & Bellstedt, 2006). Applications of HC in the range of 2 to 5% v/v to buds in table grape varieties and even in wine grapes negate the effect of endo-dormancy by producing transient respiratory disturbances and oxidative stress (Pérez, Vergara, & Rubio, 2008). Breaking of winter dormancy in buds varies with concentration, time of application, bud physiological stage and genotype (Sánchez et al., 1994). So far, HC has no commercial use in Nepal but has been used for research at 2-5% v/v in varieties like 'Steuben' and 'Cabernet Sauvignon' in past few years. Thus, this study aims to understand the current scenario of grape cultivation in Nepal and facts and speculations about application of HC in preponing budburst in grapevines for better and uniform yield. Furthermore, it will be helpful for other researches in grapevines, aiming to inspire growers and enhance the commercial viability of Nepalese vineyards.

2. METHODOLOGY

This review article relies on literatures drawn from various sources, including books, journals, conferences, proceedings, reports, theses and webpages related to viticulture. Since the researches on grapevine are limited in Nepalese context, publications are also rare. In recent years, couple of researchers are working to address the problems of grapevines in Nepalese context in particular to develop the technologies for early harvesting. Hence, key informants and the perspectives, insights from growers, experiences and information from various stakeholders have also been included into the content.

3. RESULTS AND DISCUSSION

3.1 STATUS OF GRAPEVINES IN NEPAL

In Nepal, vineyards are maintained by government farms like Directorate of Agricultural Research and the Nepal Agricultural Research Council, and some private sectors such as Kewalpur Agro Farm, The Fruits Land Nepal and so on. The estimated area under grape cultivation in these farms is about 772 Ropani (around 40 ha) with around 10,437 number of grapevines (Acharya et al., 2023).

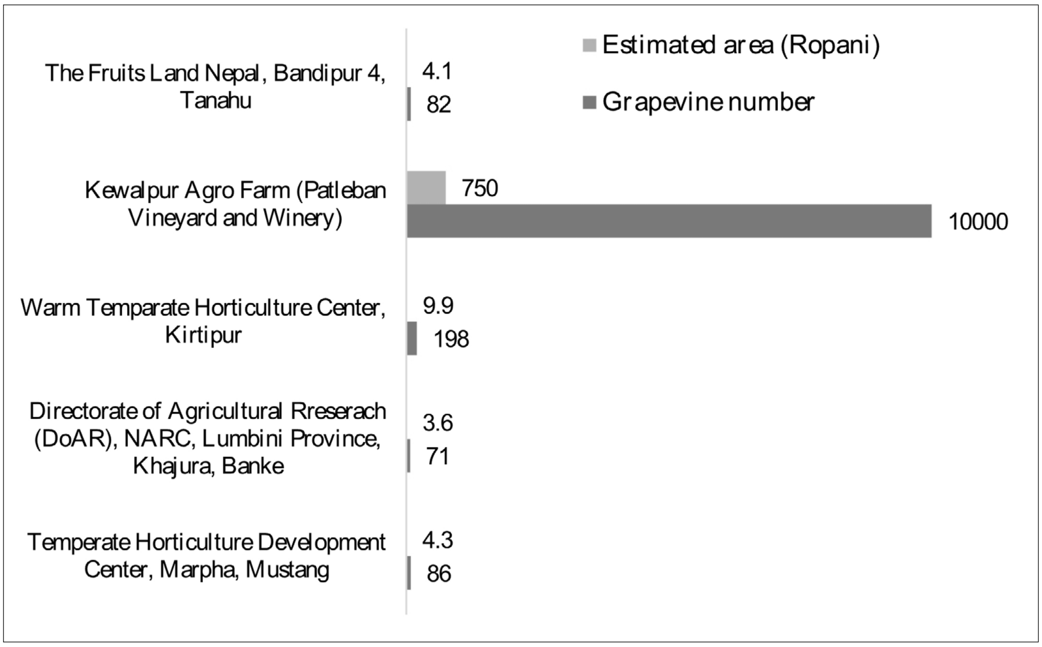

Figure 1. Status of grapevine cultivation in Nepal (Acharya et al., 2023)

3.2 GRAPE DEMAND AND SUPPLY IN NEPAL

Worldwide, grape consumption is increasing, as part of a trend towards 'convenience' fruit (small fruit that can be consumed without preparation) (Dahal et al., 2017). Grapes both table and wine enjoy modest popularity among consumers in Nepal. As people being aware of nutritional importance of fruits and some western influence on drinks, the demand of fruit including grape is increasing in Nepal. But there is always a gap between demand and supply and Nepal is trying to fulfill the gap through importing maximum of its fruit consumption which is not sustainable in long run. There was almost 8 times more import of grapes from 2014 to 2022 (Figure 2). With increasing population pressure, it is anticipated that Nepal will have increasing demand for grapes. Thus, with favorable climate and opportunities in agriculture, Nepal needs to figure out way for sustainability and giving some priorities in emerging fruit production sector as well.

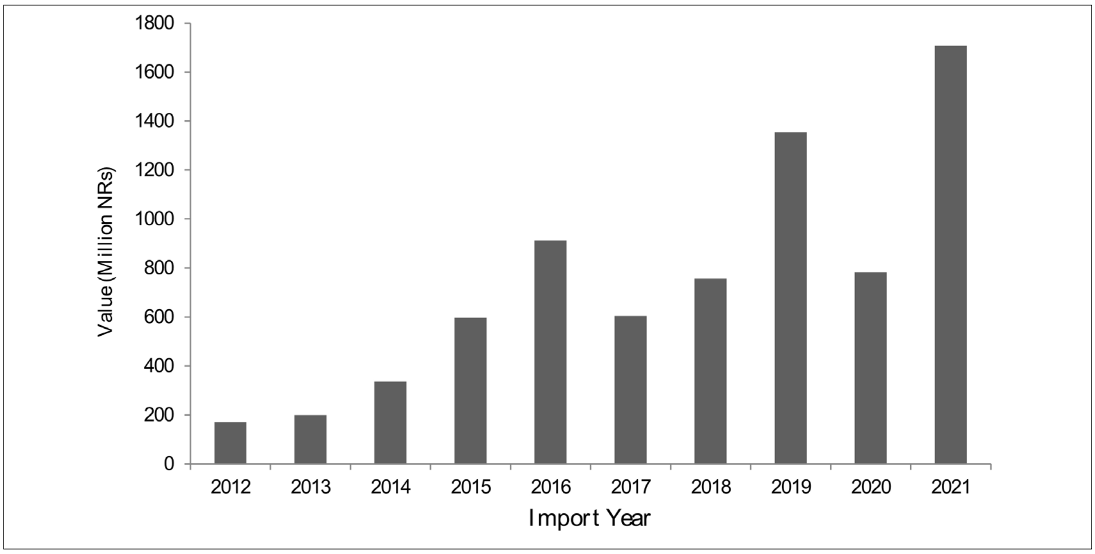

Figure 2. Import trend of fresh grape in Nepal during 2012-2021

Source: GoN (2023)

3.3 STATUS OF GRAPEVINES RESEARCH IN NEPAL

In late 1980s, several projects regarding, varietal evaluation and technology development in grapes were initiated in Nepal, particularly in Eastern Terai parts (Gotame, Gautam, Shrestha, Shrestha, & Joshi, 2020; Shrestha, 1998) but later in mid 1990s, the grape research priorities were shifted to dry subtropical western parts of Nepal (Regional Agriculture Research Station (RARS), Banke). At RARS Banke, the growth behaviors of several grape cultivars were evaluated for four successive years (Joshi, 1986). Varietal evaluation trails were performed in Warm Temperate Horticulture Centre (WTHC), Kirtipur as well, where Japanese varieties like, 'Steuben', 'Muscut Bailey A', 'Kyoho' and 'Black Olympia' were compared with other cultivars like, 'Himrod', 'Delawar', 'Olympia' and 'Tono Red' (Gotame et al., 2020). Similarly, in mid 1990s, three years varietal evaluation trial was conducted in RARS, Banke where several Indian cultivars were evaluated, out of which, Perlette and Beauty Seedless were recommended for earliness, parthenocarpic nature and good yielding characteristics (Gotame et al., 2020; KC, 1999). Miyoshi et.al. (1997) applied to GA_3 at full bloom stage on var. Himrod that had very small berry size in Kirtipur, Kathmandu reported increased berry size by 20% and 10% with 50 ppm and 100 ppm of GA_3 respectively. During 2019-2021, research were carried out to develop the innovative grape production techniques by using plant growth regulators i.e. gibberellic acid, hydrogen cyanamide and timing of pruning in different grapevine cultivars (var. Himrod and var. Steuben as table grapes and var. Cabernet Sauvignon as wine grape) in Warm Temperate Horticulture Research Centre (WTHC), Kirtipur (27°30' N latitude to 85°15' E longitude and at an altitude of 1,320 meters above sea level) and Kewalpur Agro Farm Vineyard, Dhading, Nepal (27°6` Latitude 85°6` longitude and at an altitude of 855 meters above sea level). In WTHC, Kirtipur, the HC was applied to var. Steuben and it was found that pruning followed by 2% HC application after a week was found effective leading to early and uniform budburst (Dhakal, 2021). Earlier bud burst was observed in earlier pruning followed by HC application (before December 9) and late in later application (after December 9) in var. Steuben (Dhakal, 2021).

According to Ghimire (2022) and Sapkota (2022), applying 2% HC following pruning preponed natural budburst timing and had considerable effect on growth stages, quality of budburst and quantity of budburst percentage of vine in cv. Cabernet Sauvignon in Dhading, Nepal (Ghimire, 2022; Sapkota, 2022). They suggested the optimal range of HC concentration is between 2 to 5% and the treatment preponed the budburst by 3 weeks in cv. Cabernet Sauvignon in Dhading, Nepal, also advised that the HC concentration beyond 5% led to detrimental effect in budburst, flowering and yield. Gibberellic acid application at concentration between 30 to 40 ppm when berries are around 4 mm size has been found more effective in improving quantitative measurements of grape berries and bunches especially in var. Himrod in WTHC, Kirtipur, Kathmandu, Nepal (Poudel, Atreya, & Dahal, 2022). A research was conducted on assessing the dormant bud fruitfulness by bud dissection method in var. Himrod and var. Steuben in Warm Temperate Horticulture Centre, Kirtipur which suggest bud dissection as an effective tool to analyze bud fruitfulness and predict the yield for following season (Poudel, Dhakal, Atreya, Sapkota, & Dahal, 2023). Currently, there is similar on-going research in grape bud fruitfulness and effects of different doses upto 40 ppm and time of GA application after fruit set in table grape cultivars in Kewalpur Agro Farm (Patleban Vineyard and Winery), Dhading.

3.4 GRAPEVINE IN WARMER CLIMATE

Expansion of grape production towards warmer climates is increasing since late 1990's through innovative vine management and the use of growth regulators e.g. hydrogen cyanamide (HC). The timing of key management operations (e.g. pruning, use of PGRs) are usually determined by temperature and rainfall patterns of different regions. In wet regions, a single harvest with single/double pruning is the general practice because the second harvest is not practical due to effect of rainy season on vine growth (Leao, 2014). Combinations of pruning, use of plant growth regulators (bud breaker and growth retardant) and harvesting practices alter the vine growth and dormancy in buds, hence the grape production becomes possible in wider climates around the world (Midmore, 2015). Exploration of production technologies with respect to our growing environment, topography and monsoon calendar is utmost important for the successful viticulture.

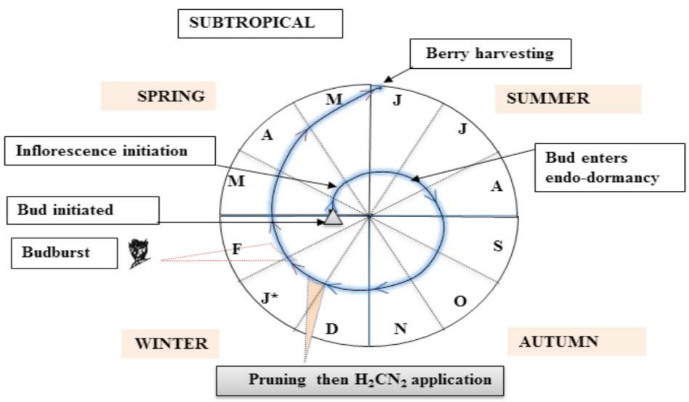

Figure 3. Reproductive cycle of the grapevine in accordance to grapevine growth stages in subtropical condition (J*- January) (Adopted from Dahal et al., 2017)

Pruning during winter season, when the vines are in dormant, is an important cultural operation to regulate vine yield. Pruning is a relatively simple method that can be used to directly limit the crop load and to select potentially fruitful buds. Response of grapevines to the timing of winter pruning and application of HC can be exploited to manipulate time of budburst and, in turn, the subsequent phenological events (Martin & Dunn, 2000). Thus, intensity as well as timing of pruning(s) and use of HC are two important practices around the world for successful vineyards in the area where the chilling temperature is suboptimal (Lavee, 2000). The main challenge in Nepalese viticulture is harvesting coinciding with the monsoon, causing significant losses. Efforts to shift harvesting earlier have been limited. A proposed solution involves advancing budburst by 20-30 days using hydrogen cyanamide after winter pruning, addressing issues related to temperature and photoperiod regulation of bud dormancy in subtropical climates as indicated in Figure 4.

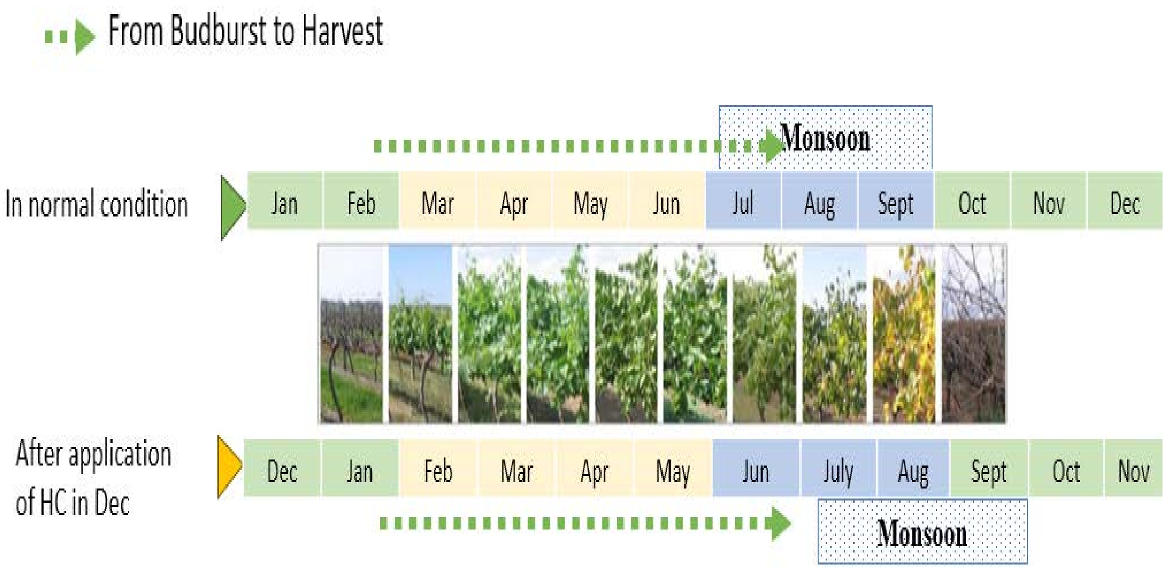

Figure 4. The lifecycle of grapevine in Nepal showing current problem and solution

3.5 APPLICATION OF HYDROGEN CYANAMIDE (HC)

Hydrogen cyanamide as bud breaker

Grapevine shows distinct response in accordance to various bud breaking agents. While standard dormancy breaking agents like Dinitro-ortho-cresol (DNOC) and thiourea have little to no effect on grapevine bud opening (Nir & Lavee, 1992), HC, popular as dormancy breaking chemical for woody perennial fruits, is the most effective in grapevines (Shulman, Nir, Fanberstein, & Lavee, 1983). Dormex® (a.i. 49% HC) is popular for its use to release buds from dormancy as well as enhancing a more uniform and rapid bud opening (Halaly et al., 2008). According to Vergara, Parada, Rubio, and Perez (2012), HC seems to induce respiratory and oxidative stress by disrupting the function of the mitochondrial cytochrome pathway, which results in the breaking of bud dormancy. However, the mode of action and interaction between respiration, cell cycle regulation and oxidative signaling has not been completely elucidated.

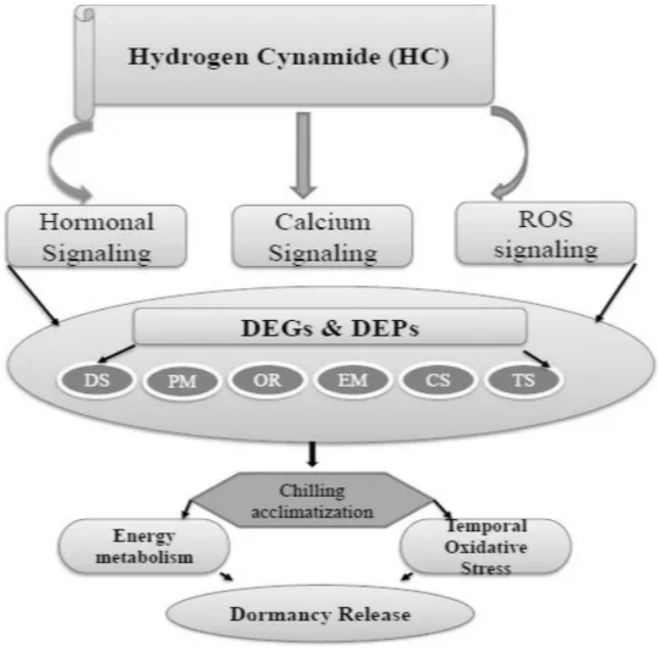

Figure 5. A proposed model of bud dormancy release in grapevine after HC application *(In this model, HC application leads to expression of genes and proteins related to hormone signaling, ROS signaling and calcium signaling which includes DS (defense and stress), PM (protein metabolism), OR (oxidation-reduction), EM (energy metabolism), CS (cell structure) and TS (transcription and signaling). Here, DEGs and DEPs implies differentially expressed genes and differentially expressed proteins respectively.)*

Source: Khalil-Ur-Rehman et al. (2019)

Hydrogen cyanamide promotes dormancy release with the association of hormone signaling, calcium signaling, hypoxia, and oxidative stress (Halaly et al., 2008; Pérez, Vergara, & Or, 2009). In accordance to Khalil-Ur-Rehman et al. (2019), HC stimulates the activity of proteins and genes involved in energy metabolism, which may cause grapevines to budburst early. Exogenous HC treatment raises the expression level of sucrose synthase, pyruvate decarboxylase, a sucrose non-fermenting (SNF)-like kinase, grapevine dormancy breaking-related protein kinase, and alcohol dehydrogenase (Ophir et al., 2009; Pérez et al., 2008). The accumulated soluble sugars provide energy for the plant to withstand low temperature in winter and maintain normal growth (Horikoshi, Sekozawa, & Sugaya, 2017). Halaly et al. (2008) mentioned HC application leads to temporary oxidative stress to release buds from dormancy. Reactive oxygen species (ROS), stimulation of the antioxidative machinery, respiratory stress, and the induction of glycolysis as a feedback effect are all brought on by transient oxidative stress (Buchanan, Gruissem, & Jones, 2015). HC temporarily elevates the level of hydrogen peroxide (H_2O_2) and rapidly upregulates certain genes associated with oxidative stress (Sudawan, Chang, Chao, Ku, & Yen, 2016), which causes a sharp decrease in catalase (CAT) activity and transient stimulation of peroxidase (POD) and ascorbate peroxidase (APX) activities that precedes the release of bud from endodormancy (Nir & Lavee, 1992; Gil Nir, Shulman, Fanberstein, & Lavee, 1986). HC enhances the budburst by Calcium (Ca^{2+})

signaling and stimulated changes in phosphorylation and transcription regulators (Pang et al., 2007). Calreticulin is a key protein involved in calcium signaling produced by modifying calcium homeostasis during dormancy regulation (Zhuang et al., 2013). The application of HC lowers the concentration of Abscisic Acid (ABA), which upturns the activity of amylase and encourages starch catabolism and soluble sugar accumulation (Liang et al., 2019). HC decreases the endogenous ABA level by promoting ABA degradation and inhibiting ABA synthesis (Khalil-Ur-Rehman et al., 2017). HC releases bud from dormancy by increasing the content of ethylene and cytokinin (Ophir et al., 2009).

Doses of hydrogen cyanamide

In subtropical viticulture, hydrogen cyanamide treatment is a popular and effective technique. On the other hand, genotypes and the timing of application (days before natural budburst or days after pruning) affect the concentration of HC. Grape varieties for table purpose should generally be treated with 2% v/v a.i. of HC with a non-ionic surfactant and a coarse droplet spray with a nozzle pressure of less than 40 psi. For temperate woody perennials, 2% HC is thought to be a nearly lethal dosage (Fuchigami & Nee, 1987). However, Siller-Cepeda, Báez, Stichez, Gardea, and Osorio (1994) found no adverse effects on grapevine growth and yield at 29° North in Mexico when grapevines were treated with up to 8% a.i. of HC at pruning or 5, 10, or 15 days after pruning. Dokoozlian (1998) suggested that the doses of HC could be increased or reduced depending on the ratio between the exposure of chilling temperature (<7°C) and chill-negating temperatures (hours >20°C). HC application has been found effective in preponing budburst by 3 weeks as compared to control in var. Carbernet Sauvignon at Kewalpur Agro Farm, Dhading, Nepal (Sapkota, 2022). The research was conducted from January 2021 to July 2021 in order to examine the effect of six different HC concentration ranging from 0-8% on budburst and yield attributes of grapevine var. Cabernet Sauvignon, among which 2% HC concentration was found as the best treatment in preponing the natural budburst time and maturity of harvest (Sapkota, 2022).

Hydrogen cyanamide application dates

Hydrogen cyanamide effectiveness on budburst depends on stage and depth of dormancy that also influence on berry maturity (McColl, 1986). In the Jordan Valley's subtropical climate, buds treated with HC sprouted 4-26 days earlier than the control, advanced flowering by 4–13 days, and produced berries with noticeably better quality than the control (Muhtaseb & Ghnaim, 2008). Earlier application of HC can advance fruit maturity by 2-3 weeks (McColl, 1986). Lavee and May (1997) mentioned that early application of HC under warm subtropical condition, advanced maturity of fruit but decreased yield due to reduced number of shoots, reduction in budburst percentage and non-uniform delayed budburst. However, in Australia's subtropical environment, HC spraying 4-6 weeks before to natural budburst greatly boosted crop production while having little to no effect on the timing of berry maturation for the cv. Muscat Hamburg variety (George, Nissen, & Baker, 1988). Moreover, it is suggested that late application especially at high concentration damage to the buds and a delay in their opening (Shulman et al., 1983).

Pruning and hydrogen cyanamide application

It is necessary to decide when and how much of the previous season's growth should be cut during the winter (dormant) pruning of grapevines to promote bud opening and preserve crop load. In the tropics as opposed to the subtropics, dormant buds respond more strongly to the pruning stimulus. In areas with low and often insufficient winter chilling, winter pruning in combination with HC treatment is crucial for controlling budburst (Lavee & May, 1997; Lombard et al., 2006). When HC is applied during the inflorescence growth stage, fruit maturity may be advanced by two to three weeks, but yield may be lowered due to reduced cluster number, smaller cluster weight, and greater floral abscission (McColl, 1986; Or, Nir, & Vilozny, 1999; Shulman et al., 1983). In cv. Flame Seedless, HC applied 1-2 weeks after pruning delayed budburst by 5 days in comparison with vines sprayed at pruning time (Siller-Cepeda et al., 1994). HC applied as early as at the pruning time in a subtropical climate of Jordan Valley enhanced budburst and maturity than that with a later application (Muhtaseb & Ghnaim, 2008). All buds in dormant shoots sprouted within 35 days in Hermosillo Valley, Mexico (Siller-Cepeda et al., 1994) and 46 days after pruning in Jordan Valley (Muhtaseb & Ghnaim, 2008). An experiment was conducted to assess the effect of different time of HC application (HC at 5% concentration was applied 7 days after pruning and pruning were done at different dates from December 21, 2021) in var. Cabernet Sauvignon at Kewalpur Agro Farm, Dhading (Ghimire, 2022). Growth stage with reference to E-L stage of vine differed significantly between treatments with higher average growth stage in early treated vine (Ghimire, 2022). Higher budburst (around 55-68% in early trimmed vines while about 10-30% in late trimmed vines) and observed fruitfulness (around 50-55% in early trimmed vines while about 10-25% in late trimmed vines) in early trimmed vines indicate a considerable impact on grapevine growth modification from the timing of pruning and HC treatment. Earlier-treated vines also had fewer days until their initial budburst which was around 1 to 10.4 days to first budburst while later-treated vines had initial budburst after 24 days of treatment (Ghimire, 2022). Moreover, several bud growth parameters reveal a detrimental effect on late-pruned vines followed by HC application, which is thought to be caused by HC's phytotoxic effect on fragile buds following their natural emergence from dormancy (Ghimire, 2022). Early sprouted buds may suffer from frost damage resulting in subsequent penalties in yield. Thus, the combination of pruning and HC application with reference to natural budburst time should be studied for effective budburst, berry maturity time and yield of the variety in a specific growing environment.

4. CONCLUSION

Grape is an emerging crop in Nepal, experiencing a consistent rise in demands. However, the production has not been much prioritized due to insufficient research efforts in viticulture. The major challenge limiting grape production is excessive rainfall (monsoon) coinciding with the fruit harvest period. Despite of climatic potentiality of grapes cultivation in Nepal, the production window is short. Thus, winter pruning with HC application for artificial induction of bud dormancy release, emerges as a plausible strategy to prepone bud burst and achieve early maturity. Moving ahead, more research must be done to determine the timing and dosage of HC in early maturing varieties and given growth condition to maximize its efficacy in bud burst and maturity. Through careful vine management procedures, Nepal may overcome the obstacles caused by monsoon-related problems and fully reap the benefits of grape farming, improving the country's agricultural landscape and satisfying the growing demand for grapes in the area.

DECLARATION

The authors declare no conflict of interest.

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