Proceeding of
2nd International
Conference on
Horticulture
Godavari, Lalitpur
3-4 April 2023

TECHNICAL PAPER

Understanding Bud Fruitfulness and Importance of Gibberellic Acid (GA3) Application(s) in Successful Grapevine Cultivation

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Abstract

During the Hill Agriculture Project 1976-1979 AD and Horticulture Development Project 1985, germplasms of grapevines were officially introduced in Nepal. Grape farming is considered the remunerative enterprises among the fruit crops. During the last five years 2017-2021, the import value of fresh grapes and dried grapes increased by about 181 and 200 percentage respectively. The huge gap in demand and production shows the great scope to expand both area and production of grapes. However, the monsoon period coinciding with berry harvest is considered a major constraint for its successful cultivation. This paper aims to develop understanding the facts of bud fruitfulness and the exogenous application of Gibberellic Acid (GA) at different berry growth stages along with hydrogen cyanamide (H_2CN_2) application for better and uniform budburst and production in designing research and developmental activities. The burst bud is just the visible phenomenon, however the fruitfulness is the qualitative phenomenon. The mechanism is a complex process leading to the formation of reproductive structures having three well-defined stages (formation of anlagen-inflorescence primordia-flowers). Better and uniform budburst is found to be dependent both on concentration and time of H_2CN_2 application during later winter. GA is used to cluster loosening, thinning and increasing berry size for better yield and quality of grapes. GA used to improve the berry size is a common practice, however, its doses and stages of application determines the successful viticulture. The strategy needed to develop the GA doses and stages of application are discussed and a plausible strategy is recommended.

Keywords: Berry size, bud fruitfulness, dormancy, gibberellic acid (GA syn. GA_3), grapevine

Introduction

Grape (*Vitis vinifera* L.) cultivation is one of the most remunerative farming enterprises among the fruit crops. It is believed to have been started the Grapevine cultivation within the Rana regime (>70 years ago). Although small vineyards on government research stations as well as farms were established in temperate and warm temperate climates from 1968 AD, viticulture has not been prioritized and there is effectively no commercial table grape production in Nepal (Dahal et al., 2017). During the Hill Agriculture Project (FAO) 1976 to 1979 and Horticulture Development Project, 1985, many germplasms of temperate fruits, grapevines were officially introduced and maintained in Kirtipur, Jumla and Mustang. Demonstration and extension programs were implemented around Kathmandu valley and its vicinity (Shrestha *et al*., 2017).

Grapes are one of the largest fruit crops and table grapes are one of the major types of grapes grown worldwide, which are the fresh consumed grapes. The demand for table grapes is growing globally, particularly in Asia-Pacific, the Middle East and Russia (MI, 2022). In Nepal, grapes enjoy modest popularity among fruit consumers. Figure 1 shows that during the last five years, the import value of fresh grapes was increased by about 181% (6,12,852 in 2017 to 17,20,593 NPR'000 in 2021), likewise, the import value of dried grapes increased by about 200% (86,246 in 2017 to 2,58,559 NPR'000 in 2021). With the data, annual demand of fresh as well as dried grapes is estimated to increasing by more than 30% in Nepal. Thus, there is crucial to increase both area and production of quality grapes in Nepal not only for fulfill the national demand but also to meet the national policy of import substitution.

However, heavy rainfall coinciding with the fruit harvest period is considered a major constraint for the commercial production of table grapes in Nepal. Poor yield and monsoon associated diseases are identified as the major limitations to successful grape production in western terai (Joshi, 1986; Shrestha, 1996; Shrestha, 1998). Dahal *et al.* (2017) also reported that the poor yield and early monsoon were identified as major problems, innovative vineyard management technique has yet to practice in Nepalese viticulture to uphold grapevine cultivation. Exogenous application of GA_3 has various roles on yield attributes (including improvement in berry size) of grapevine. The doses and frequency of GA_3 application are very specific to the variety as the seed content in berries varies with cultivars. Some absolute seedless cultivars are less responsive to 50 ppm of GA_3 while 5 ppm is detrimental to the seeded cultivars (Dokoozlian and Peacock, 2001). Indeed, application of GA_3 in grapevine has not been reported so far in Nepal. Therefore, cultivar specific researches are necessary to identify the doses and application timing in a given vineyard management practices for better yield and quality of grapes.

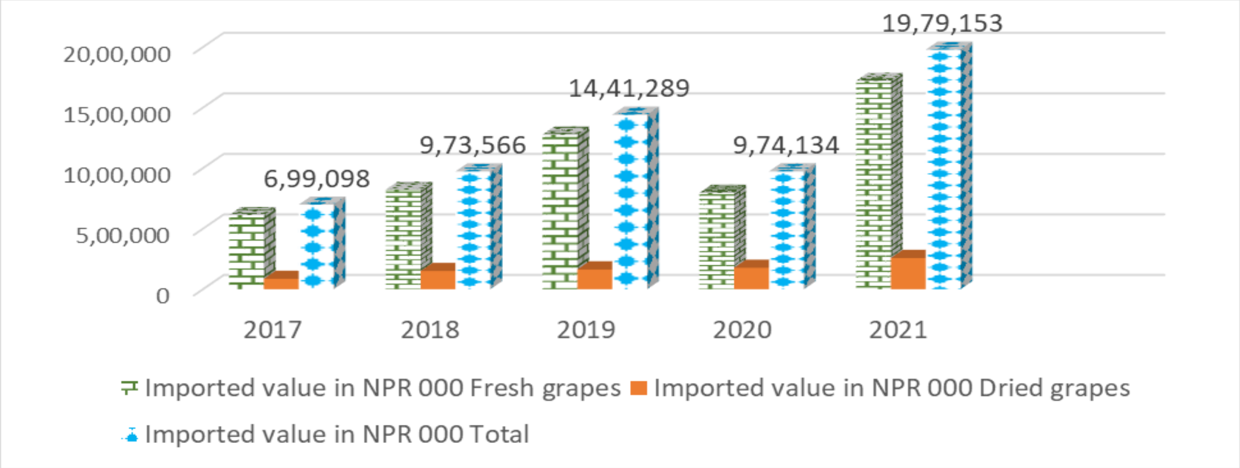

Figure 1. *Import trend of fresh and dried grapes in Nepal during 2017-2021 (TEPC, 2022).*

Therefore, this paper aims to develop better understanding about the facts of bud fruitfulness and the application timing and doses of Gibberellic Acid (GA_3) along with Hydrogen Cyanamide (H_2CN_2) in designing research and developmental activities for better yield and quality. This information will be also applied in the ongoing innovative field research in the existing commercial vineyard to encourage to the grower and to make the Nepalese vineyard competitive.

Materials and Methods

The paper is based on literature review from different sources such as journals, conferences, reports, web pages and books related to viticulture. Key informants' views and information and experiences of various stakeholders were also included in this paper.

Results and Discussion

Major cultivation of grapes in Nepal

In Nepal, established vineyards were limited to small area in government farms and research centers such as Directorate of Agricultural Research, (DoAR), Nepal Agricultural Research Council (NARC), Khajura, Banke; Warm Temparate Horticulture Center, Kritipur and Temperate Horticulture Development Center, Marfa, Department of Agriculture (DoA) and farms under different private sectors such as Kewalpur Agro Farm of a Patleban Vineyard and Winery and The Fruits Land Nepal, Bandipur, Tanahu. Major varieties grown in Nepal were Himrod, Muscat Belly, Beauty Seedless, Perlette, Stuben, Punjab Purple, Thompson Seedless, Pione Selection, Summer Black, Fuji Minori, Seedless Black, Talizman, Polonskei Muskat, Ontario, Arkadia, Cabernet Sauvignon, Regent, Kyoho, Campbell Early, Delaware, Buffalo, etc. Based on the information available, total number of grapevines (Ca. 10437 vines) in the aforementioned vineyards is calculated and the total area (772 ropani) occupied is estimated in table 1.

Table 1. Cultivation status of grapes in Nepal

SN	Farm	DoA/NARC/ Private	Variety	Grapevine	Estimated area (Ropani)
1	Temperate Horticulture Development Center, Marfa, Mustang	DoA	Himrod, Muscat Belly	86	4.3
2	Directorate of Agricultural Research, (DoAR), NARC, Lumbini Province, Khajura,Banke	NARC	Beauty seedless, Perlette, Stuben, Punjab purple, Himrod	71	3.6
3	The Fruits Land Nepal, Bandipur 4, Tanahu	Private	Himrod, Stuben, Thompson Seedless, Pione Selection, Summer Black, Fuji Minori, Seedless black	82	4.1
4	Kewalpur Agro Farm (Patleban Vineyard and Winery)	Private	Different table and wine varieties: Talizman, Polonskei, Cabernet Sauvignon, etc.	10000	750
5	Warm Temparate Horticulture Center, Kritipur	DoA	Himrod, Stuben, Muscat Belly, Kyoho, Campbell Early, Delaware, Buffalo	198	9.9
			Total	**10437**	**771.9**

Bud fruitfulness in grapevine

Bud and its dormancy

In the grapevine, a hierarchy of buds is present in each leaf axil, forming a complex referred to in many languages as an 'eye'. The true axillary bud, the 'prompt bud', opens sometimes to become a 'lateral' shoot, growing concurrently with the main shoot during spring and summer. Its basal bud is the 'dormant bud', 'winter bud' or 'latent bud' which hardly ever opens during the season of its formation, but only at next spring. It contains at least two more buds within its scales. The French system of numerating these organs (e.g. Bugnon and Bessis, 1968) helps to clarify this hierarchy: N is the main shoot, N+1 the prompt bud/lateral shoot, N+2 the latent bud (which under normal conditions forms next year's shoot), N+3 any lower-order buds which will usually open only if the N+2 bud fails to open due to unfavorable events (e.g. bud necrosis) and which form together with the N+2 the nodal 'eye' (Figure 2). The complex of the buds N+2 and N+31, 2 is enclosed by the bud *scales and forms the nodal 'eye'* (Lavee and May, 1997).

Figure 2. *The axillary organs of the grapevine shoot N (Keller, 2015)*

N+1 = axillary shoot (often called the lateral), developing from the prompt bud; N+2 = main latent bud; $N+3_{1,2}$ *= secondary latent buds* (Lavee and May, 1997).

Berkowitz *et al.* (2014) illustrated that normal developmental transitions from bud dormancy to budburst, to flowering and fruit initiation are in synchrony with seasonal changes and their disruption can impact on grape production. Bud dormancy is an important survival strategy in perennial plants including grapevine with key regulators of dormancy being temperature, especially accumulated chilling time and photoperiod (Carmona *et al.*, 2008). The dormant bud remains in a state of dormancy until the following year due to hormonal inhibition of the apex of lateral shoots in subtropics and temperate regions (Magalhães and de Viticultura, 2015; Jackson, 2014). Anatomically, the dormant buds comprise a large central bud, which corresponds to a primary bud and two smaller buds (secondary and tertiary buds) on either side of the primary. Due to their complexity in structure and having three different buds within a structure, dormant bud is also defined in the literature as compound bud.

After the formation of reproductive structures dormant buds go into dormancy until next spring when they restart their growth in response to environmental conditions and complete their development with the formation of flowers and berries (Carmona *et al*., 2007, 2008; Srinivasan and Mullins, 1980b; Vasconcelos *et al*., 2009).

Primary bud necrosis (PBN)

Primary bud necrosis (PBN) is a physiological disorder occurring in the compound axillary buds of grapevines. PBN causes the axillary bud to senesce and in some cases secondary buds can also abort.

In the grapevine bud, if the primary bud does not survive or is unable to develop, the secondary buds enlarge and produce shoots to compensate for the loss of the primary bud (Lavee *et al*., 1981; Naito *et al*., 1986). Although little information is available on the fruitfulness of secondary buds, it is widely observed that secondary shoots produce less and small bunch/berries than primary shoots. On occasion secondary buds have also been affected by bud necrosis (Naito *et al*., 1986; Morrison and Iodi 1990; Wolf and Warren, 1995). The incidence of PBN was found to depend on cultivar and viticultural practices (Collins *et al*., 2006). Susceptible cultivars include Queen of the Vineyard (Ziv *et al*., 1981), Flame Seedless (Morrison and Iodi, 1990), Riesling (Wolf and Cook, 1992), Viognier (Wolf and Cook, 2000) and in Australia the most susceptible variety is Shiraz (Dry and Coombe, 1994; Collins and Rawnsley, 2004). A number of different stresses are reported to be responsible for the occurrence of PBN, e.g. excessive shoot vigour (Lavee *et al*., 1981; Dry and Coombe, 1994), canopy shading (Perez and Kliewer, 1990; Wolf and Cook, 1992; Wolf and Warren, 1995), high levels of gibberellins (Ziv *et al*., 1981) and low carbohydrate levels associated with shading (Vasudevan *et al*., 1998a, b).

Bud dormancy release

Chilling

Many studies have been made to examine the effect of chilling on releasing the buds of deciduous plants including grapevines from the state of dormancy. Indeed, it is generally accepted that chilling is essential to terminate this state of dormancy and to allow normal budburst (Lavee and May, 1997). According to Pouget (1988), a period of at least seven consecutive days with a mean daily temperature below 10°C is needed for this to occur in cv. Merlot.

The requirement for chilling may not be obligatory for breaking dormancy in all grapevine varieties and that chilling has a quantitative rather than a qualitative effect. In its absence and without other dormancy-breaking measures, grapevine buds will show limited, uneven and delayed budburst. Their chilling requirement is low as compared to that of peaches (Lavee and May, 1997).

High temperature response

Breaking dormancy in grapevines may be affected by supra-optimal high temperature (Pouget, 1963). High temperature may either prevent the onset of dormancy or replace the effect of chilling by activating an alternative pathway (Lavee and May, 1997).

Bud scale removal

Several reports indicated that the removal of bud scales fosters budburst (Antcliff and May 1961; Iwasaki and Weaver, 1977; Iwasaki 1980; Mizutani *et al*., 1985). It has been reported, but not proven, that this effect is related to abscisic acid being present in the scales (Lavee and May, 1997).

Cyanamide

As bud dormancy is a quantitative state, suboptimal chilling conditions in sub-tropical regions will lead to poor bud burst with subsequent yield penalties. This often needs intervention by application of chemicals such as hydrogen cyanamide (HC i.e. chemically H_2CN_2) to increase and synchronise budburst (Halaly *et al.*, 2008).

Grapevines are unique in their response to dormancy breaking agents. Grapevines were found to be most responsive to calcium cyanamide ($CaCN_2$) (Iwasaki and Weaver, 1977). HC was found to be the active ingredient in $CaCN_2$ (Shulman *et al*., 1983). The mode of action and optimal time of application has been studied extensively during the last 15 years in almost all grape-producing countries of the temperate, subtropical and tropical zones and the substance gave similar beneficial results wherever tried (e.g. Shulman *et al*., 1983; Lin *et al*., 1983; Lin, 1984; Lavee *et al*., 1984; Luvisi, 1984; Williams and Smith, 1984; Fuchigami and Nee, 1987; Yang *et al*., 1990; Paioli-Pires *et al*., 1993). Budburst in grapevines (and also fruit trees) induced by HC was found to be dependent both on concentration and time of application (Lavee *et al*., 1984; George *et al*., 1988; Fuchigami and Nee, 1987). Late application especially at high concentrations may result in damage to the buds and a delay in their opening.

Generally, the natural bud break occurred in the second half of February. The early application was given 9-10 weeks before natural bud break and the late application 5-6 weeks before natural bud break (Or *et al*., 1999).

Flowering and Bud fruitfulness

Grapevine reproductive development extends over two growing seasons (vegetative cycles), for the complete formation of inflorescences and clusters. Induction and floral differentiation, the mechanism that leads to the formation of reproductive structures inside dormant buds, is a complex process divided into three well-defined stages (formation of anlagen, inflorescence primordia and flowers). This sequence of stages comprises morphological, biochemical and physiological events, influenced by a set of environmental and endogenous factors. Inflorescence primordia formation determines the potential number of clusters that will be formed in the following growing season (Monteiro *et al*., 2021).

Similarly, reported that flower formation in grapevines follows three well defined steps (Watt *et al*., 2008): (i) anlagen or uncommitted primordia, are formed in the apices of latent buds on shoots of the current season; (ii) these specialized meristematic structures may differentiate inflorescence primordia (IP); and (iii) individual flowers are formed on IP (Perold, 1927; Barnard, 1932; Barnard and Thomas, 1933). For grapevines grown in temperate climates, steps 1 and 2 are completed during the previous season. Individual flowers, on the other hand, are not formed until during budburst (BB) in the current season (Barnard, 1932; Snyder, 1933; Winkler and Shemsettin,1937; Scholefield and Ward, 1975; Srinivasan and Mullins, 1981).

Determined by the differentiation of anlagen in inflorescences during the first vegetative cycle, bud fruitfulness represents the first measure of productive potential, as it defines the number of bunches that will be formed for the following season (Dry, 2000; Ferrer *et al*., 2004; May and Antcliff, 1973; Srinivasan and Mullins, 1980a). Thus, bud fruitfulness provides an estimate of the potential yield for the following season (Collins *et al*., 2020; Dry, 2000). It depends on the variety, type of bud, position of the bud and climate during primorida development along the shoot whose effect manifests in terms of the number of inflorescences per bud and size (number of flowers) (Dahal *et al*., 2019; Magalhães and de Viticultura, 2015; Ramos, 1991). Buds are considered fruitful when they have at least one primordium inflorescence. Conversely, the bud is considered infertile in the absence of inflorescence primordia or the existence of only tendril primordia and leaves (Srinivasan and Mullins, 1980b).

Factors affecting induction and flower formation

Fruitfulness is determined by simple visual observation and counting the number of inflorescences in the young shoot (Monteiro *et al*., 2021). Forcing budburst of dormant buds is a simple and expeditious method, which does not require detailed knowledge about the anatomy of buds. However, the results are not immediate, as is necessary to wait for the development and visualization of inflorescences (Clingeleffer *et al*., 2001).

Different studies have focused on the environmental (abiotic) and endogenous (biotic) factors that directly and indirectly influence the process of induction and differentiation of inflorescence primordia (Buttrose, 1974; Carmona *et al*., 2008; Khanduja and Balasubrahmanyam, 1972; Li-Mallet *et al*., 2016; Srinivasan and Mullins, 1980b; Vasconcelos *et al*., 2009). Temperature, light, water status and macronutrients availability are the environmental factors that most influence these processes. In addition, endogenous factors such as carbohydrate reserves (source/sink regulation), hormonal balance and genetics also have an important role (Carmona *et al*., 2008; Li-Mallet *et al*., 2016; Vasconcelos *et al*., 2009). Thus, positive stimuli during the differentiation of anlagen will promote the inflorescence primordia development and have decisive impacts on the fruitfulness (Monteiro *et al*., 2021).

Crop load adjustment

Winter pruning is a first viticultural practice through which yield can be regulated and quality improved (Reynier, 1990). Each year, during dormancy, the bud load is adjusted according to the bud fruitfulness in order to meet the productive objectives (Zhu *et al*., 2020). However and remarkably, grapevine reproductive development extends over two vegetative cycles (growing seasons). It begins with inflorescence primordia formation in first year and with differentiation of the flowers, development of the clusters until the physiological maturation of berry and seeds in following year (Magalhães and de Viticultura, 2015).

Ferrara *et al*. (2014) reported that berry size is genetically predetermined among cultivars, but it can be considerably increased by crop load adjustment (Dokoozlian *et al.,* 1994), bunch and berry thinning, trunk girdling (Dokoozlian *et al*., 1994) and the use of plant growth regulators (PGRs).

Application of GA

There are a number of synthetic PGRs, some of which are copies of the endogenous forms, others affect the action of endogenous PGRs or their receptors. The use of gibberellic acid by the table grape industry to control berry size is an example of the utility of PGRs in manipulating fruit development. PGR treatments were applied at low concentrations by spraying in the presence of a surfactant (Davies *et al*., 2014).

In many varieties of the grapevine, as in many other deciduous species, application of GA during the previous growing season will delay and even completely inhibit bud opening in the following growing season. The relationship between GA and dormancy has been studied by Allewedt (1961) who found that exogenous GA applied during dormancy development and dormancy had little effect on the time of budburst, but that applications during the latter were inhibitory. Delays in budburst after GA applications were also noted by Weaver (1959); Eris and Çelik (1981); Iwasaki (1980). Indeed there are many reports that in many but not all varieties GA applications during flowering in one season lead to complete failure of buds to burst in the next one (Lavee and May, 1997). However, application of GA in grapevines has following implications:

Control berry size

Early application of GA is a known inducer of shot berry formation due to: (1) GA-induced shot berry development is mediated by induction of parthenocarpy, (2) the parthenocarpic nature of the shot berries is responsible for their inability to develop to the size of the stenopermocarpic berries on the same cluster, as complete lack of endogenous GA. Interestingly, GA application did not lead to significant increase in shot berry size in such mixed clusters (Or *et al*., 2014).

The use of PGRs is common in table grape viticulture (either seeded or seedless grapes) and more limited is their application for wine grape cultivars. Gibberellins (GAs) are commonly applied to many table grape cultivars. The mechanism of GAs is the stimulation of cell elongation and division, with higher sugar and water intake into the cells. The compound generally used is GA isomer, which is commonly applied after berry-set to increase berry size (Dokzoolian and Peacock, 2001). To achieve increase in berry size, two separate applications of 7.5g within the same season are required: first application of 7.5g at smallest berry size (4 mm diameter) and second at larger berries up to 6 mm diameter (ProGibb, 2016).

Trunk or cane girdling consists of removing a ring of bark (phloem tissue) from either the vine trunk or canes to restrict the movement of assimilates from the aerial portion of the vine to the roots in order to increase the berry size (Dokoozlian *et al*., 1995). The PGRs improved berry size but induced a slight change of the skin colour which resulted in a pale yellow-green; some differences were also detected in the metabolic profile (Ferrara *et al*., 2014).

GA sprays and cincturing are used by the table grape industry to increase berry size of seedless varieties because of consumer demand for larger berries. Interrogation of the historical weather data suggested that most of the problem was associated with heat stress during the time when GA is applied for berry sizing. On average, maximum temperatures were 5-10°C higher during late November in the 3 seasons when widespread berry collapse was observed, compared to other seasons when there was no or very low incidences of berry collapse (Clingeleffer *et al*., 2014).

Thinning berry

Thinning berries is an important cultural operation in table grape production, in order to eliminate the excessive number of berries, maximising the quality and value of the production (Domingos *et al*., 2014). The reduction of total berry number and removal of small-sized berries results in improved final bunch aspect (decreasing bunch compactness, increasing berries weight and size and uniformising the maturation within the bunch), quality (sugar and colour pigments accumulation) and decreased incidence of diseases (Di Lorenzo *et al*., 2011).

The most common thinning practice is chemical thinning by GA sprays followed by hand adjustments when necessary. However, the success of GA treatment depends on the environmental conditions, the concentration and time of application varies with the cultivar and its use is not authorised in organic production and, in some countries, in integrated crop management system (Domingos *et al*., 2014). To achieve berry thinning, application of GA should be done when bloom or blossom is at 100% capfall stage (Sultanas var) or 70% (Perlette var) depending on variety (ProGibb, 2016).

Cluster loosening

GA bloom application is commonly used in table grapes as a means for inducing cluster loosening, however the environment is known to play a key role in the response to growth regulator treatments (Domingos *et al*., 2014). The same cultivar can show different results over the years, as observed for 'Sovereign Coronation' in a three-year trial (Reynolds *et al*., 2006). In 'Thompson Seedless', the lower GA concentration successfully increased the flowers and berries drop, in contrast with higher doses, which agrees with similar reports for 'Crimson Seedless' (Dokoozlian and Peacook, 2001).

Conclusions

Normal developmental transitions from bud dormancy to budburst, to flowering and fruit initiation are in synchrony with seasonal changes and their disruption can impact on grape production. Yield of grapes can be regulated and quality improved thorugh the crop load adjustment practice. Better and uniform budburst is found to be dependent both on concentration and time of H_2CN_2 application. The early application of H_2CN_2 might be given 9-10 weeks before natural bud break and the late application 5-6 weeks before natural bud break. Buds are considered fruitful when they have at least one primordium inflorescence. Bud fruitfulness provides an estimate of the potential yield for the following season. GA is used to cluster loosening, thinning berries and increasing berry size for better yield and quality of grapes. However, the timing and stages of application are crucial. For increasing berry size, two separate applications of 7.5g within the same season are required: first application of 7.5g at smallest berry size (4mm) and second at larger berries up to 6mm. This plausible strategy needs to be incorpoated in the planning and implementation of grapes production.

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