

Influence of Hydrogen Cyanamide (50% S.L.) on uniform sprouting, yield and quality in grapes

Abstract

Grape (*Vitis vinifera* L.) is considered as one of the most important commercial fruit crops in temperate, tropical and subtropical regions of the world. In India grape cultivation in tropical and subtropical regions assumes great significance, but one of the biggest issues preventing grapes from being produced in warm climates has been the lack of or insufficient winter chilling. Under these circumstances, there is uneven bud bursting, flowering, and delayed fruit development. The most crucial component of grape development is, consistent bud breaking is required to increase grape quality and maturity. Many plant growth regulators are used to promote bud burst and increase the proportion of bud break. Several locations across the world utilize plant growth regulators to intentionally cause bud break. The application of Hydrogen Cyanamide (H_2CN_2) during pruning time of grapes, helps to enhance growth uniformity, encourage earlier and more even bud break, raises the proportion of fruit buds, berry set, number of clusters per vine, weight of clusters, and dimensions of clusters. It also gives positive response to various metabolic activities; maximize yield and Maturity, Fruit quality, as well as production efficiency. In grape orchards, it is commonly used to promote consistent and improved bud burst, shortened flowering period, and boost output.

Keywords: Hydrogen Cyanamide, Bud break, Sprouting, Fruit quality, Flowering.

1. Introduction

Grapes (*Vitis vinifera* L) are one of the most important fruit crops for local consumption and export. It is considered as most popular and favorite fruit in the world, because of their excellent flavor and high nutritional value [1]. Grape is commercially originated in the temperate zone and has adapted to sub-tropical and tropical agro-climatic conditions prevalent in Indian sub-continent. Grapes are consumed fresh, used to make wine, and used as a source of important nutrients and antioxidants (84.7 million tons). Grapes are made up of water (81%), 18% carbs, 1% protein, and very little fat, and the output of grapes is utilized to make wine in about 71% of cases, fresh fruit in 27% of cases, and dried fruit in 2% of cases. According to Food and Agricultural Organization (FAO) grapes are grown over 75,866 square kilometers [2].

The China (11,200.00 MT), Italy (8,149.40 MT), the Spain (6086.92 MT), United States (5,488.47 MT), are the top four grape-producing nations in the world in terms of production. India stands on 7th position with (3,358.00 MT) production [3]. Grapes are in high demand not only in India but also in other markets, thus in order to meet this demand and boost exports, it is required to raise the production of grapes of a standard grade in accordance with importer demands [4].

In India the major grape growing states are Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. Maharashtra is leading state in area and production of grapes in the whole

country. Total Area under grapes in Maharashtra is 86 thousand ha and production is around 774 thousand tons of grapes annually [5]. The production of grapes is crucial to the Indian agro-economic system, yet every year farmers experience significant yield loss as a result of biotic and abiotic stressors[6].Uniform bud sprouting during October pruning is thought to be fundamental and crucial among the several operations in tropical and subtropical conditions [7].

A major reason for low productivity of grapes in tropical countries is a poor bud break. Bud breaking is an issue that only occurs in India in a number of circumstances because a cold environment is a necessity for grape production. One of the most significant problems limiting the output of grapes in warm areas, though, has been the absence of or inadequate winter chilling, Unequal bud bursting, prolonged flowering, and delayed fruit development is occurs in these conditions [8]. Uneven budburst, especially on vines with high levels of vigor, may limit the number of cordon spurs that form and hence decrease output. The number of spurs may not rise significantly if the cordon length is increased each season. In tropical and subtropical climates, several horticultural techniques like defoliation, withholding irrigation and fertilizer, and severe pruning have been employed successfully for management of the sprouting in grapes. However, these techniques are time consuming, expensive, and labor-intensive. In those conditions, for increasing quality and maturity of grapes uniform development of bud breaking is needed. Earlier and uniform bud break is most important factor for overall development of grapes [9].

To encourage bud burst and enhance the percentage of bud break, a various plant growth regulators are utilized. The Chemical plant growth regulators are used in several places of the world to artificially induce bud break. Potassium nitrate, ethephon, thiourea, paclobutrazol, Hydrogen Cyanamide were used for increasing bud break in grapevine. Among this growth regulators, Hydrogen Cyanamide (H_2CN_2) reported best result for bud break. The application time and concentrations employed determine the outcomes of Hydrogen Cyanamide (H_2CN_2). Mostly application of Hydrogen Cyanamide (H_2CN_2) should be applied at pruning time of grapes. It is an effective plant growth regulator that works in conjunction with chilling to promote earlier and more even bud-break, increase yield, and improve growth uniformity [10]. Additionally, Hydrogen Cyanamide (H_2CN_2) increases fruit buds percentage, berry set, cluster per vine, cluster weight, and cluster dimensions. It also gives positive effect on main shoot length and pushed harvest dates [11].

2. Why uniform bud break is needed

The grapevine exhibits continuous vegetative growth in tropical temperature zones, with no break in photosynthetic activity, allowing for production at any time of the year and the achievement of at least two yearly seasons. In the process of adapting to environmental conditions, fruit plants in temperate climates go through dormancy stages of grapevines. As a result, bud dormant stage poses a management challenge in areas with subtropical or hot winters, typically leading to heterogeneity in bud bursting, flowering, and low fruit development. The accumulation of cold hours is the natural catalyst that encourages the overcoming of the dormant stage in the buds; however, in regions where the amount of cold

hours is less than what the grapevine needs, it is necessary to use physiological effect products in an effort to break the vegetative inactivity state. There are numerous substances, including thiourea, potassium nitrate, ethephon, paclobutrazol, and calcium cyanamide, have been used to encourage the breaking of buds in tropical grapevines. However, it has been found that Hydrogen Cyanamide (H_2CN_2) is the most effective, encouraging favorable effects in the plant's productive features, particularly in the flowering percentage, and, as a result, in the crop yields [12]. In table grapes a lack of winter chilling might cause unequal bud bursting, prolonged flowering, and delayed fruit development. Uneven budburst, especially on vines with high levels of vigor, may limit the number of cordon spurs that form and hence decrease output. The number of spurs may not rise significantly if the cordon length is increased each season. In those conditions, for increasing quality and maturity of grapes uniform development of bud breaking is needed. Earlier and uniform bud break is important factor for overall development of grapes [9]. Under warm conditions of Egypt, Red Roomy grape cultivar's producers are most concerned with the delayed and erratic bud break. Due to May's extremely high temperatures, bud break occurred later than normal and irregularly, which resulted in lower fruit set than anticipated. Numerous deciduous fruit crops have inconsistent and delayed bud break, which is caused by a lack of cooling hours needed to complete bud break. In warm weather, artificial means are greatly needed to make up for the lack of natural chilling requirements and achieve an economically viable production of dessert grapes. In many different grapevine varieties, Hydrogen Cyanamide (H_2CN_2) utilized to speed up the termination of bud dormancy, improve and regulate bud burst, and boost yield and berry quality [1]. Grapevines undergo two pruning cycles every year in tropical climates, after harvesting and during the summer time is when the initial pruning is carried out. In warmer regions, bud breaking is a major obstacle for uniform bud burst. Uneven and erratic bud sprouting slows future cultural activities [13].

3. Effect of Hydrogen Cyanamide on Duration and Uniform bud break in grapevines

The vines treated with Hydrogen Cyanamid, which recorded significantly higher percentage of bud break and short duration of bud break compared to untreated vines. The Higher percentage of bud break was recorded on third year indicated that there is a uniform bud break and no depression due to continuous use of Hydrogen Cyanamide (H_2CN_2).

Table 1: Effect of Hydrogen Cyanamide (50% S.L) on Bud break

Treatment	Bud break				
	T1	T2	T3	T4	Mean
Year 1	99.2	97.2	93.6	24.4	78.6
Year 2	78.0	85.6	86.4	26.0	69.0
Year 3	94.4	96.0	92.0	56.8	84.8
Mean	90.5	92.9	90.7	35.7	

In comparison to the untreated control (T4), vines treated with Hydrogen Cyanamide (H_2CN_2) showed considerably higher rates of bud break. Regarding the years, the notable differences were also reported. The third year had the greatest amount of bud break. This shows that the continues usage of Hydrogen Cyanamide did not cause any depression in bud break. Among the various treatments, Hydrogen Cyanamide @20 ml per liter (T1) had reported a greater rate of bud break (99.2%) in the first year.

Table 2: Effect of Hydrogen Cyanamide on duration of bud break

Treatment	Duration of Bud break				
	T1	T2	T3	T4	Mean
Year 1	2.2	2.7	2.9	12.2	5.0
Year 2	4.4	3.9	4.3	10.5	5.8
Year 3	6.6	4.2	4.8	14.2	7.5
Mean	4.4	3.6	4.0	12.3	

In terms of year, the Hydrogen Cyanamide treatment and the length of bud break showed substantial changes but their interaction did not show any differences. First-year bud break lasted for a shorter period of time. The first and third years did not show any distinct differences. The evident from these results reported that the time of bud break was observed to be reduced more effectively in treatment treated with Hydrogen Cyanamide @30 ml /litre than other treatment[14].The bud breaking percentage by application of 2 % (v/v) aqueous solution of Hydrogen Cyanamide at three different times,dormant (D), swollen (S), and open (O). These phenological growth stages were assessed twice, on February 20 and March 4. In one vineyard, the amount of final bud break was measured at the conclusion of the growing season.On February 20, vines treated on January 1 (A), January 9 (B), or January 18 (C), respectively, had 10%, 52%, and 53% of their buds open, whereas vines not treated had only 4% of their buds open. OnFebruary 20, similar percentages of budbreak were produced by treatments B and C. On the other hand, on March 4th, the latter treatment had the greatest success rate (77% vs. 61% budbreak). While untreated vines had less than 24% of their buds open on March 4th, these two treatments had 50% bud breaks on February 20th. In contrast to the control, application of Hydrogen Cyanamide on January 9 or 18 speed up bud growth and advanced bud break by at least 13 days [15].

Table 3: Percentage of Buds at each of the first three phenological stages at two different dates. The vines were either sprayed with water (control) or with a 2 % (v/v) aqueous solution of Hydrogen Cyanamide on the 1 (A), 9 (B) or 18 (C) January.

Treatments	20 Feb			04 Feb			FB
	D	S	O	D	S	O	
Control	71.6a	24.7d	3.7c	14.2a	61.7a	24.0d	80.2a
A	36.7b	54.2a	10.1	10.9ab	52.3b	36.8c	82.0a
B	13.0c	35.2c	51.9a	8.1b	30.7c	61.2b	83.7a
C	0.5d	46.9b	52.6a	0.3c	22.9c	76.8a	88.1a

The Hydrogen Cyanamide either SBB (Sangh bud break 50% S.L.) encouraged uniform sprouting in “Thompson Seedless grapes”. The data (Table 4) showed that the administration of Hydrogen Cyanamide (SBB 50% S.L.) over the untreated vines resulted in substantial variations in percent bud break. Due to the application of 3 % SBB, the highest rate of bud break (81.0%) was seen.

Table 4: Effect of Hydrogen Cyanamide (H₂CN₂) on bud breaking in “Thompson Seedless” grapes.

Treatment	Conc. Used (%)	Bud sprouting (%)	Duration of bud sprouting from initiation to completion (days)	Days taken for bud sprout from pruning
T0 @untreated	-	31.58	9.28	16.16
T1 @ SBB 50% S.L	0.5	70.03	4.09	11.50
T2 @ SBB 50% S.L	1.0	74.33	3.84	11.16
T3@ SBB 50% S.L	1.5	75.20	3.80	10.80
T4 @SBB 50% S.L	2.0	78.33	3.63	10.83
T5 @SBB 50% S.L	2.5	80.08	3.51	10.33

T6@ SBB 50% S.L	3.0	81.00	3.20	10.81
SEm(±)	-	(1.80)	(0.20)	(0.56)
C.D.@ 5%	-	(5.41)	(0.61)	(1.69)

Date of pruning	Temperature	
	(Max) °c	(Min) °c
10-10-2006	32.4 °c	12.3° c
14-10-2007	34.3 °c	15.0° c

In terms of bud sprouting duration, all treatments were noticeably better than the untreated vines. The use of Hydrogen Cyanamide as compared to the untreated control, the difference in the number of days for bud sprouting was considerable after pruning. As the concentration of Hydrogen Cyanamide (SBB) grew, it took fewer days for the buds to sprout [7]. The application of 1% Hydrogen Cyanamide on 10 years old “Askari grapes” trees enhances bud break by 7.78% and 6.67% within 20 to 30 days [16]. Application of Hydrogen cyanamide at 3.0 percent reported that the length of the bud sprouting process was cut in half, from 9.28 days to 3.20 days, as opposed to the untreated control. The untreated control took the longest amount of time (16.16 days) to sprout compared to all SBB treatments (10.33 to 11.50 days) [17]. The experiment on three years old seedless cultivars (ARRA15: white variety, ARRA18: black variety and ARRA19: red variety) were grown in open-field conditions or under greenhouse. 12 days after pruning, 3.5% Hydrogen Cyanamide (H₂CN₂) solution was administered and twelve days later, buds were wiped with cotton dipped in the Cyanamide solution. Hydrogen Cyanamide (H₂CN₂) spray raised budburst percent by over 74% under greenhouse conditions and by roughly 31% in open-field conditions, with no discernible cultivar-specific changes. Furthermore, the application of Cyanamide considerably increased the average dry weight of shoots, primarily in ARRA15 (by 59%) and ARRA18 (by 49%) in open-field conditions, as well as in all cultivars in greenhouse conditions (by around 68%). For ARRA19, the treatment greenhouse with Cyanamide had the highest dry weight of shoots ever measured. When evaluating the effects of different cultivation methods, it was found that non-treated plants' budburst was higher in open fields than in greenhouses, but the contrary was true after Hydrogen Cyanamide application. Furthermore, Hydrogen cyanamide (H₂CN₂) only increased bud development in open-field with no discernible impact on budburst uniformity, where as it induced a more uniform and full budburst (100%) under greenhouse (approximately 27 buds on the 6 bud positions). In general, under greenhouse conditions, blooming and fruit set dates were earlier than in open-field conditions. Additionally, all treated cultivars' budburst and harvest dates were pushed by one week in a greenhouse [11].

Earliest application of Hydrogen Cyanamide (H_2CN_2) able to achieve an earlier budbreak and a larger yield without affecting the final berry size or the period until fruit maturity. Climate factors, such as sudden temperature swings following the use of chemicals to break up rest, can also have a deleterious impact on the bud-break process. Depending on the plant variety, timing of treatment, application rate, stage of bud development, method of application, latitude, and weather conditions, Hydrogen Cyanamide (H_2CN_2) has produced varying results in grapevines. Even on the same variety, it may have no effect on bud development or promote, delay, or kill buds depending on the concentration and time of application. There have been reports of grapevines treated with Hydrogen Cyanamide (H_2CN_2) displaying earlier and more uniform budbreak, flowering, ripening, and approaching maturity as well as having higher fresh weight of the fruit than the control [18]. Thompson Seedless vines, treated with either 2% or 4% of Hydrogen Cyanamide (H_2CN_2) at 53 and 67 days before anticipated budbreak were compared to untreated vines. Early application of 4% Hydrogen Cyanamide (H_2CN_2) accelerated fruit maturity by 17 days and improved budbreak. However, the period of maturity was only accelerated by 4 days when the same concentration was applied 53 days before natural budbreak. In comparison to the control, the proportion of budburst increased significantly with each treatment. Hydrogen Cyanamide (H_2CN_2) improved fruit quality but had no appreciable impact on yield. The treatment yielded a greater index of maturity than 67 days. As compared to untreated vines, all treated treatments caused a more uniform budburst. The first spray using the higher concentration (4%) produced the best uniformity [19]. The grapevines shows irregular and delayed budbreak, fewer shoots and clusters per vine, and uneven fruit development due to insufficient winter chilling. As a result, fruit yield and quality are decreased. It is frequently difficult to get enough cooling in this area for typical budbreak because the autumns and winters are warm. In order to solve this issue, farmers spray Hydrogen Cyanamide (H_2CN_2) on the vines just after pruning. Hydrogen Cyanamide (H_2CN_2) expedites budbreak and enhances the uniformity of budbreak in grapevines grown in low-chill environments [20].

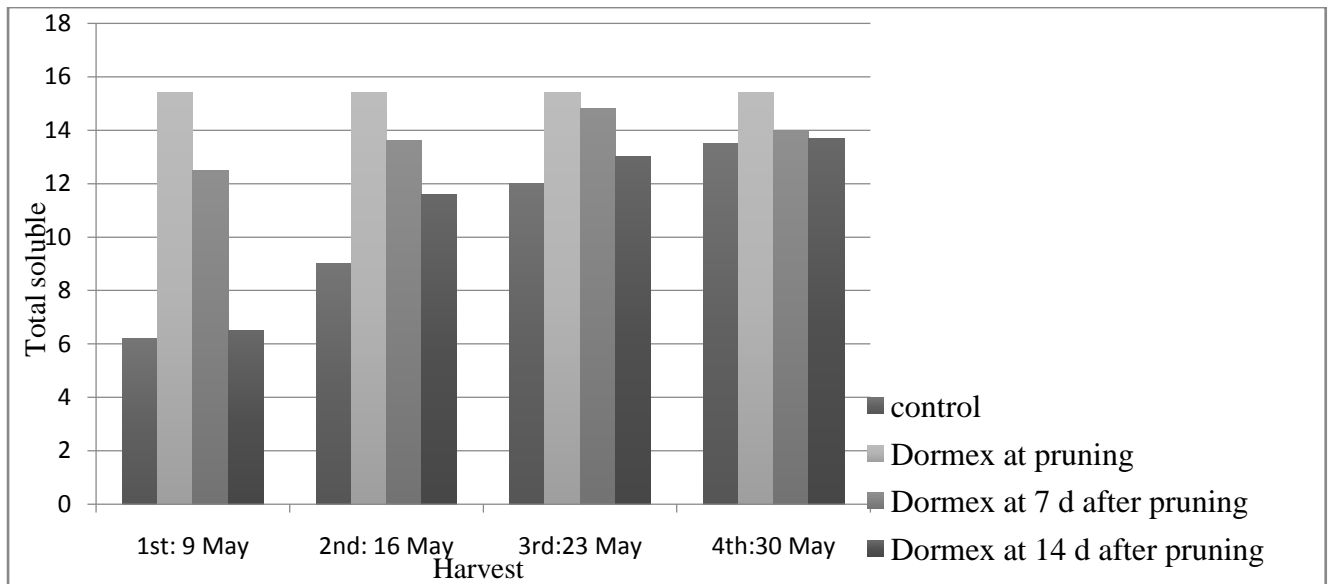
4. Effect of Hydrogen cyanamide on Yield, Quality and Maturity of grapes

4.1 Yield and Quality

When Cyanamide was given in late July almost six weeks before the start of natural budburst and almost 3-fold increase in yield on young, vigorous "Muscat Hamburg" grapevines. Depending on the cultivar, the total bunch production of vines treated with cyanamide was significantly higher than the untreated control. With each year Hydrogen Cyanamide (H_2CN_2) was applied, bunch production on the treated vines rose, which was correlated with spur production on the treated vines, a higher number of shoots produced per spur, and a larger number of bunches produced per shoot on spurs. On fresh shoots started on the cordons, some bunches were also formed. The greater number of spurs produced per vine was linked to a more regular bud break of latent buds on the cordons [9]. The Hydrogen Cyanamide (H_2CN_2) is active ingredient was sprayed on canes of pruned vines immediately after pruning in flame seedless grapes. According to the results of study data showed that pre-harvest treatment of Hydrogen Cyanamide and 100 ppm ABA helped to expedite ripening and increase fruit quality in grapes [21]. The yield of Perlette grape vines has also been

significantly enhanced by the treatment of Hydrogen Cyanamide (H_2CN_2). Under the Ludhiana conditions, vines treated with Hydrogen Cyanamide produced the highest mean yield (42.0 clusters per vine), followed by 41.5 and 40.0 clusters per vine treated with 2.5 and 1.5% Hydrogen Cyanamide. The uniform and greater bud burst in treated vines may be the cause of the increased number of cluster spur vines found in Hydrogen Cyanamide treated vines compared to untreated control vines. Similar to Bathinda circumstances, vines treated with Hydrogen Cyanamide at 2.0% had a greater output in terms of clusters per vine, followed by vines treated with Hydrogen Cyanamide at 2.5 and 1.5%, which recorded 75.5 and 69.0 clusters per vine, respectively. In comparing to untreated vines, the application of Hydrogen Cyanamide soon after pruning accelerated the times of flowering and ripening by 5-0 days and 4-5 days respectively. The first week of January marked the time for pruning, which resulted in a higher yield (31.4 kg) than the untreated control. Under Ludhiana conditions, the vines treated with 1.5% Hydrogen Cyanamide recorded the heaviest bunch weight (389.5 g), but they did not substantially differ from other treatment and control vines. Contrary to the results stated above, vines left untreated had a maximum bunch weight of 363.0 g, which was comparable to vines treated with 2.0 and 2.5% Hydrogen Cyanamide at Bathinda circumstances [22]. The fruits of the treated vines had significantly reported the highest fruit weight, total soluble solids (TSS) and the Titratable acidity (TA) and it was the only treatment that exceeded the TSS level. Additionally, higher TSS and lower TA were observed for fruits of Hydrogen Cyanamide (H_2CN_2) treated vines during all harvest dates in comparison with the untreated vines. The study showed that, treated vines of Hydrogen Cyanamide recorded good maturity and quality of fruits. Dormex (H_2CN_2) should be applied as early as at pruning time to obtain early bud break and maturity. In addition, more TSS was detected from vines treated with Hydrogen Cyanamide (H_2CN_2) throughout every harvesting date as compared to the control. However, the TSS level of the fruit was modest or unaffected by delaying Hydrogen Cyanamide (H_2CN_2) treatment for longer than two weeks after pruning [18].

Figure 1: Effect of application of Dormex on fruit total soluble solids of grape cultivars.



Source: Muhtaseband Ghnaim[18]

The application of Hydrogen Cyanamide (H_2CN_2) enhance the quality of bunches per shoot, Berry weight and diameter. In fact, vines treated with Hydrogen Cyanamide (H_2CN_2) tended to have fewer unopened buds and weaker shoots, which should increase overall leaf area and photosynthetic activity and lead to improved berry weight and diameter. In order to speed up and homogenize better fruit quality (size) and earlier maturity may result, which should allow for higher fruit prices [23].

4.1.2 Fruit Maturity

The cardinal grapes grown in unvented, unheated vegetable glasshouses were tested to see how well weekly pruning procedures and applications of Hydrogen Cyanamide (H_2CN_2) from early May to late June advanced budburst, blooming, coloration, and maturity. Compared to untreated vines that were trimmed on the same day, plants treated with Hydrogen Cyanamide matured about a month earlier. With the treatment of Hydrogen Cyanamide, budburst took place 28–61 days after pruning. The earliest application and highest concentration of Hydrogen Cyanamide (H_2CN_2) produced best results. The maturity gain was less noticeable when Cyanamide was used closer to spontaneous bud break. An earlier and shorter flowering phase results in early fruit maturity. The treated vines with Hydrogen Cyanamide matured around one month earlier than untreated vines [24]. Hydrogen Cyanamide (H_2CN_2) at A 25 mg/ml was sprayed to Sultana H4 and Cardinal wine vines in the scorching subtropical desert of Central Australia immediately following pruning on May 2, May 30, June 27, June 25, July 25, and 16, 12, and 8 weeks prior to the typical time of budburst, around August 22. Both cultivars responded to Cyanamide, and 2–4 weeks after administration, the vines burst into bud. Sultana H4 fruit ripening was accelerated by 4, 3, and 2 weeks by Cyanamide sprays on May 30, June 27, and July 25, respectively. By using Cyanamide on June 27 and July 25, respectively, Cardinal's ripening was brought up by 3.5 and 2.5 weeks, respectively. The region's commercial table-grape output is significantly impacted by the capacity to accelerate maturity. Applying Cyanamide between mid-May and mid-August, it is possible to promote the budburst of field-grown table grapes in the

subtropical Australian climate, which can advance the maturity of the fruit by as much as 4 weeks. The fruit produced by the vines treated in this way is of the highest quality [25]. Under lack of chilling conditions, it's necessary to use artificial rest-breaking agents such as Hydrogen Cyanamide (H_2CN_2), which is best for grapevines. However, the timing of application affects the treatment's efficacy. Two trials were conducted in mild winter conditions. In both laboratory and field testing, application of Hydrogen Cyanamide (H_2CN_2) was most efficient in accelerating and increasing bud break. Additionally, this application boosted berry weight and diameter and accelerated fruit maturity. Our research showed that, despite its reliance on the timing of administration, Hydrogen Cyanamide was effective in increasing budbreak and fruit maturation in Superior Seedless table grapes [15].

4.1.3 Carbohydrates content

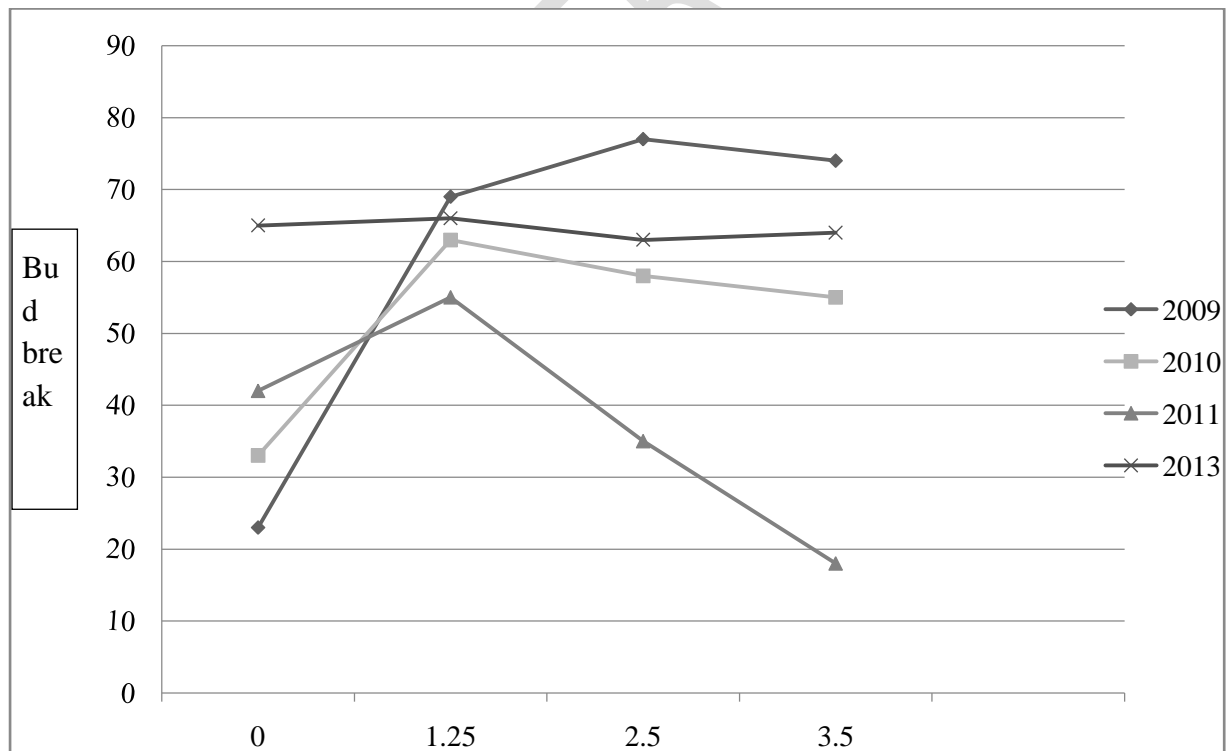
The buds treated with Hydrogen Cyanamide had maximum sucrose levels (42.66 mg/g DW) after 96 hours. At 7 days, higher fructose contents (33.50 mg/g DW) were detected in comparison to the control; Hydrogen Cyanamide also enhanced the Total soluble solids (TSS) contents. When compared to control buds, buds treated with Hydrogen Cyanamide (H_2CN_2) at 96 h had higher TSS contents (42.66 mg/g DW) [26].

5. Effect of different doses of Hydrogen cyanamide on grapes

The bud opening was improved in pruned perlette vine by applying Hydrogen Cyanamide (H_2CN_2). The most successful concentrations were 0.25 to 0.63 M, which after 30 days produced 80% opened buds. The variety "Dan Ben Hanna" required 1.25 M, to produce equivalent results. On perlette vines, the Hydrogen Cyanamide solutions had a stronger effect, with using 0.25 M Cyanamide caused 72% of the buds to open after 30 days. The outcomes were better when the compounds were employed at higher doses (1.25 M) [27]. The six treatments of Hydrogen Cyanamide (H_2CN_2) doses taken in experiment are 0, 10, 20, 30, and 50 ml L⁻¹. The 'Niagara Rosada' sprouted more widely after the application of Hydrogen Cyanamide, resulting in an almost 14-day head start over the control. The treatment of 20 ml L⁻¹ resulted in the highest sprouting percentage. The treatments did not differ statistically for the quality and productivity-related measures [28]. Hydrogen Cyanamide (H_2CN_2) treatments significantly affect bud burst, shoot length, number of leaves, and leaf area, but do not significantly affect root number or length. Due to the fact that it takes the shortest amount of time to break shoots, the longest shoots generate the most leaves, and the concentration (20 ml/l) is recommended for breaking the dormancy of grape cuttings. The concentrations of Hydrogen Cyanamide treatments are significantly different to bud burst, shoot length, number of leaves, and leaf area, but not significantly different to number of roots and root length. The treatment treated with Hydrogen Cyanamide at concentration 40 ml/l was showing shoots flush fastest that in 6 days after planting. It is advised to use Hydrogen Cyanamide at a concentration of 40 ml/l to promote the growth of grape cuttings since it quickens the growth of the shoots [29]. The several experiments were conducted in commercial vineyards using the cultivar Perlette during the production cycles of 2009, 2010, 2011, and 2013. To determine the impact of increasing doses of Hydrogen Cyanamide (H_2CN_2) on shoot buds and grape cluster production. Hydrogen Cyanamide concentrations of 0, 1.25, 2.5, and 3.5%

(p/v) were examined. The number of clusters per plant and the final bud were counted to determine the impact of the treatments. The control treatment without the application of Cyanamide had the lowest percentage, ranging from 25 to 66%, while the Cyanamide treatments had percentages of budding higher than 50%. In the year 2009 reveals that the maximum percentage was observed in the Cyanamide treatment at a dosage of 1.25 percent, which was statistically different from the concentration of 3.5%. This finding suggests that high doses of Cyanamide restricted bud sprouting. There were no statistically significant differences between the treatments in the data from the final bud of 2010. According to data from 2011, the sprouting levels in the treatments and the fact that the treatment with the highest bud level above 65% was the one with the dose of 1.25% Cyanamide were both statistically significant at the 2.5% and 3.5% treatments as well as at the control level. The data of the final sprout level of cycle 2013 are presented, the curve corresponding to this cycle, shows that the sprouting levels in the different Cyanamide treatments are close to 80%, but higher and statistically different to the Cyanamide-free control, which presented levels close to 25%, confirming that the Cyanamide product induces sprouting of the grapevine buds [30].

Fig 2: Sprouting level (%) with different concentrations of Hydrogen Cyanamide (H₂CN₂) in cv.perlette in the years cycles 2009, 2010, 2011, and 2013.



Source: Martinez-Diaz *et al.*, [30]

Concentration of Hydrogen Cyanamide (%)

6. Biochemical changes associated with using Hydrogen Cyanamide

The experiments conducted on, "Thompson seedless" vines were pruned on three distinct dates, 18 October, 31 October, 15 November in order to create variation in bud break. At each pruning, half of the vines received a consistent 15 % Hydrogen Cyanamide (H_2CN_2), while the other served as untreated vines. The interaction of pruning time and Hydrogen Cyanamide treatment had significant effect on peroxidase activity. The treated buds recorded significantly more protein than the untreated ones. The interaction between Hydrogen Cyanamide application and day's protein estimation was significant. The amount of total sugar available in treated buds was less as compared to control buds. The interaction effect between pruning dates and the application of Cyanamide was recorded for the total sugar available in the buds. The results showed that high enzymes activity, protein synthesis, and sugar utilization were all involved in the metabolic process of sprouting [31]. The treatment of Hydrogen Cyanamide (H_2CN_2) induced the unregulated expression of sucrose synthase, sucrose phosphate synthase. The treated group's soluble sugar content grew quickly and was higher than that of the control vines, but the starch concentration was significantly lower in the Hydrogen Cyanamide treated group than in the untreated ones. In the Hydrogen Cyanamide treated group, the concentration of indoleacetic acid (IAA) and zeatin (ZT) increased, whereas the concentrations of abscisic acid (ABA) and gibberellin (GA) declined [32]. A catalase activity was decreased in the samples treated with Hydrogen Cyanamide (H_2CN_2) solution (1%) as compared to the untreated buds, and hydrogen peroxide accumulated in the buds throughout the same period. In samples treated with Hydrogen Cyanamide (1%) throughout the bud break release period as compared to the initial sampling date, putrescine concentration increased more than six times, but spermidine and spermine concentration decreased significantly. Buds treated with Hydrogen Cyanamide also had higher levels of total nitrogen concentration in buds due to rise in amino acid concentrations [17].

6.1 Enzymes activity

6.1.1 Change in ascorbate peroxidase activity- In contrast to catalase activity, APX (Ascorbate peroxidase) activity was significantly stimulated after Hydrogen Cyanamide (H_2CN_2) administration. In fact, APX activity in the treated buds grew quickly from 0 to 5 day peaking at roughly 120% on day 5 of forcing, before declining to low levels for the forcing rest period. On the other hand, after 10 days of forcing, APX activity in untreated buds dropped by 19% and reached its lowest point. After that, it stayed at this low level continuously [23].

6.1.2 Changes in peroxidase activity-It started to rise right away and peaked on day five at roughly 158% of its starting activity. After then, with the foundation of budbreak, this activity dropped quickly, reaching its lowest point near the end of forcing. POD (peroxidase) activity dropped over the first three days of forcing in untreated buds before remaining constant for the remainder of the observation period. After receiving Hydrogen Cyanamide, (H_2CN_2) there were brief changes in POD and APX activity, and after 5 days, recovery set in. The patterns of activity changes for the two enzymes were comparable, but the magnitude of the rise varied. Peroxidase activity increased more rapidly [23]. Hydrogen Cyanamide (H_2CN_2) results in rapid cellular hypoxia with the release of liberated Cyanamide on aerobic respiration of catalase activity, and induction of H_2O_2 generation, administration. Several

enzymatic systems, including NADPH oxidase, amine oxidase, nitrate reductase, and arginine-nitric oxide synthase, are thought to be responsible for the rapid production of reactive oxygen species, and nitric oxide (NO). Genes involved in antioxidant defense systems are activated by signaling molecules H_2O_2 and ethylene. To prevent Prolonged cell death (PCD), the antioxidant machinery and associated pathways (such as FSD, POD) are activated. Cell wall loosening and expansion are brought on by H_2O_2 inside the cell wall. Additionally, has a role in signaling, defense, and anabolic pathways as well as in the creation of other ROS. In the presence of H_2O_2 and O_2 , OH loosens the cell wall, and H_2O_2 also promotes the peroxidase-mediated cross-linking of the structural components of the cell wall. ROS causes the production of ROS sensitive genes in the signal transduction pathway. Ca_2^+ channels, a signal transduction pathway, and protein kinase activity are all activated by H_2O_2 and OH. These reactions control the pathways that produce and scavenge ROS by activating various transcription factors. Finally, these transcription factors regulate the expression of downstream functional genes that allow grapevine buds to emerge from dormancy and resume growth [33]

ROS: Reactive oxygen species.

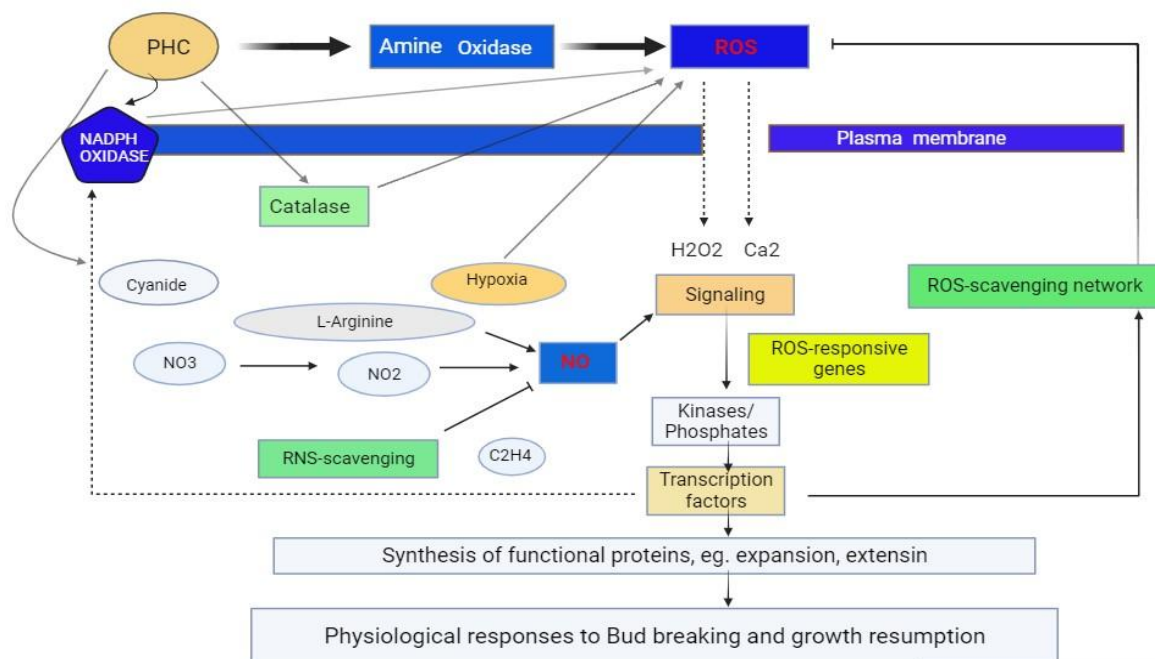
OH: Hydroxide.

APX: Ascorbate peroxidase

PCD: Programed cell death.

NO: Nitric oxide.

Fig 3: Physiological response to bud breaking and growth resumption.



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Source: SudawanB.*et al.*, (2016)

7. Concluding Remarks And Proposal For Future Research

The application of Hydrogen Cyanamide has shown promising results in enhancing grape cultivation by promoting uniform sprouting, improving yield, and enhancing fruit quality.

The use of Hydrogen Cyanamide can lead to earlier bud break, more consistent growth, and ultimately earlier fruit maturity. These improvements can have a significant impact on grape production, allowing for better fruit quality, increased market value, and potentially higher profits for grapes growers. To further explore the potential benefits and mechanisms of Hydrogen cyanamide in grapes cultivation, future research could focus on investigating long terms effect of Hydrogen Cyanamide application, molecular and physiological responses, different application methods of Hydrogen Cyanamide, exploring influence of environmental factors on efficiency of Hydrogen Cyanamid in grape cultivation.

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