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Anatomical study and natural incidence of primary bud necrosis and its correlation with cane diameter, node position and sampling date in *Vitis vinifera* L. cv. Askari

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ABSTRACT

This study was conducted to determine the correlation between the natural incidence of primary bud necrosis (PBN) with cane diameter, node position and sampling date. Compound buds at nodes 1 to 20 from mature canes with diameters of >10 mm and < 10 mm were dissected and assessed for the presence of PBN. Results indicated that the effect of cane diameter, node position and date of sampling as well as interaction of diameter×node, diameter × date and node×date on the incidence of PBN were significant. However, the highest percentage of PBN (19.3%) was observed on thicker canes and the lowest (7.74%) was observed on thinner canes. From the viewpoint of bud position, nodes from 1 to 5 and from 16 to 20 on canes had the highest (39.57%) and lowest (0.2%) PBN, respectively. Also, the highest of PBN% was observed in February (18.09%) and March (18.78%). The interaction showed that there was a positive correlation between thickness of cane and nodes proximal position on percent of PBN. When sampling date was delayed, the incidence of PBN was greater in proximal nodes than distal ones. Results of the present study showed that PBN is an important factor responsible for low fruitfulness in vineyards under consideration.

Keywords: Askari, Bub necrosis, Cane, Node position, Vigour, Vitis vinifera. L

INTRODUCTION

A large number of grapevine cultivars are cultivated in Iran that Askari is one of the major table grape cultivars. Bud necrosis with its significant effect on yield reduction is a common phenomena in some Iranian vineyards and also within several cultivars in different parts of the world [2, 1, 12, 14, 13, 16, 6, 21].

The grapevine bud contains three individual buds. The main central bud is termed ‘primary’ and on either side of this bud are the ‘secondary’ and ‘tertiary’ buds. Generally, the primary bud develops into a new fruiting shoot in spring, while the secondary and tertiary buds remain dormant. If the shoot of the primary bud is damaged or dies, the secondary buds may develop a shoot to compensate for the loss. The death of the primary bud is termed primary bud necrosis. In this situation, secondary buds may burst, they often bear no fruit or produce smaller bunches resulting in yield loss. The disorder usually affects the primary buds, but occasionally the secondary buds will also abort[17].

Bud necrosis (BN) of grapevines is a physiological disorder of the compound axillary buds [2, 9, 1, 12, 14, 11, 13, 16, 6, 21]. In some countries the incidence of PBN in vineyards have been investigated and reported that this physiological disorder significantly reduces yield. Bud necrosis was reported from different parts of the world, including Australia [6], California [13], Chile [16], India [1, 2], Japan [14] and Virginia [21]. High shoot vigour, Shade, excessive irrigation [21], low bud carbohydrate [18] and high gibberellic acid levels [22] have all been associated with bud necrosis.

Cane vigour, which can be quantified as cane diameter, internode length and growth rate, is often positively correlated with BN [6, 12, 21]. Lavee *et al.* [12] reported that in the cultivar of 'Queen of Vineyard', canes with diameters of >10 mm typically showed 15 to 50% more BN than did canes of < 10 mm diameter. Similarly, Dry and Coombe [6] in Australia working with Shiraz cultivar also reported that thicker shoots (>12mm) had 15 to 40% more BN than did thinner shoots (<12mm) from nodes 2 to 7. Additionally, Wolf and Warren [21] found that BN incidence of Riesling was positively correlated with specific growth rate of shoots measured in the three-week period after flowering. It should be emphasized, however, that these reports have only shown correlations with BN incidence, that cause and effect relationships are lacking, and that conflicting data also exist. Dry [5] showed that the level of PBN is directly proportional to the severity of shoot topping, defoliation or shoot thinning. Severe shoot thinning increased the incidence of PBN in Shiraz, whereby removal of shoots promotes increased vigour of remaining shoots. However, studies in Chile showed that a modest level of shoot thinning reduced the incidence of bud necrosis in Sultana [16], while in Riesling the effect of shoot thinning on the incidence of PBN was variable between seasons [21].

Though the vigorous growth of the shoot can be detected visually but their quantitative measurement and its relation with yield reduction both in different regions and different cultivars seem to be necessary. Low bud fruitfulness and low percentage of bud burst are the most important problems in some Iranian vineyards. Dissecting buds in autumn can raise awareness of PBN and fruitfulness of bud in the following spring, Hence, growers should determine the level of bud necrosis prior to winter pruning so that pruning techniques might be adjusted to account for the bud damage. The aim of this research was to determine the existence of bud necrosis, its percentage in Askari grapevine and its relationship with vine vigour (cane diameter), bud position, and sampling date.

MATERIALS AND METHODS

Bud samples were taken from Askari grapevine at Sisakht vineyards, south-western of Iran (latitude 30^{o}, 51$^{/}$, 57$^{//}$ North, longitude 51^{o}, 27$^{/}$, 24$^{//}$ East, 2200 m above mean sea level) from November 2007 to March 2008. The mean daily maximum and minimum temperature for Sisakht zone were 28.8 ^{o}c and -13.6^{o}c respectively. The vines were 18-year-old on own roots, trained to a head system. Plants were spaced 2.5 m apart on rows 3 m apart. The vines were pruned to 60 buds. All cultural practices were applied uniformly across blocks and in accordance with standard commercial practices. The experimental design was a randomized complete block with four replications. The sampling dates were 21- Nov, 21- Dec, 20- Jan, 19- Feb and 19 March. Cane diameter was measured at the mid-point between 1st and 2nd nodes. The samples were placed in sealed plastic bags and stored in a cool place (4 ^{o}c) to minimize water losss. Compound buds at node 1 to 20 from the mature canes with diameter >10 mm and canes of <10 mm diameter were grouped: 1-5, 6-10, 11-15 and 16-20 nodes, dissected and assessed for the presence of PBN. The presence of PBN was readily determined by making a transverse cuts with a scalpel at half the height of the bud; additional cuts were used to check the state of the secondary buds. The cuts were made deep enough to ensure that the three buds (primary, secondary and tertiary) to be exposed. Buds were dissected under a Binocular microscope at 10-40x magnification n and a digital microscope (Dinolite-AM413T) was used for taking photos. A bud with no damage would show green tissue for the primary, secondary, and tertiary bud, indicating that these buds are alive. In contrast, a damaged bud will show a dark/brown discoloration as illustrated in Fig.1. The collected data were subjected to analysis of variance.

Statistical analysis

Data were analyzed using SAS package program(SAS Inc. Raleigh, Nc. USA) and mean were compared by Duncan,s mulltiple Range Test at $P \leq 0.01$. Correlation co-efficients were calculated for relating PBN to the cane diameter, node position and sampling date.

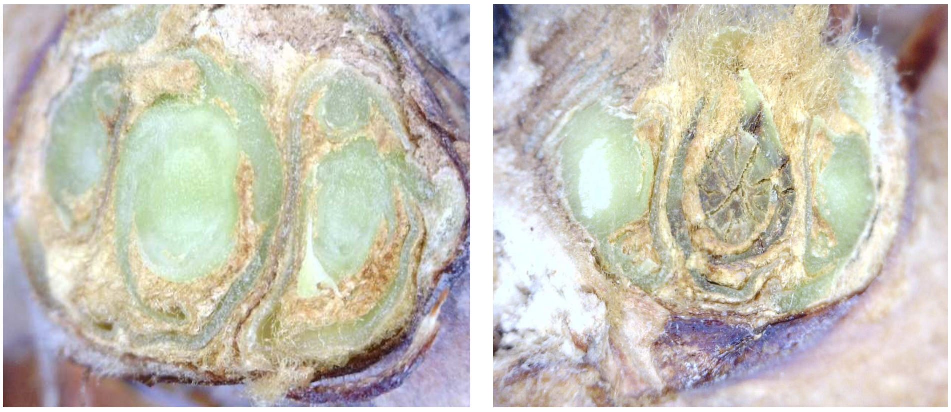


FIGURE 1 Cross sectioned compound bud showing all buds are alive (A), and damaged primary bud (B) in Askari grapevine.

RESULTS

The results indicated that the effects of sampling date, cane diameter and node position and the interaction effects (diameter × node, diameter × date and nod × date) on %PBN were significant at 1% level of probability (Table 1).

Table 1. Anaalysis of variance results of cane diameter, node position and date sampling on %PBN

Sources	Df	Mean squre	Sig.
Cane diameter	1	5359.225	**
Node position	3	13158.283	**
Date sampling	4	907.756	**
Diameter×Node	3	1406.342	**
Diameter×Date	4	96.756	*
Node×Date	12	418.460	**
Diameter×Node×Date	12	50.644	ns
Eror	120	36.879	

, indicates means are significantly different at 5% and 1% level, respectively*

Results also showed that PBN was highest (19.31%) in canes with diameters of greater than 10 mm and lowest in canes with smaller than 10 mm diameter (Fig. 2). Therefore, a positive correlation was found between cane diameter and %PBN (Fig 8).

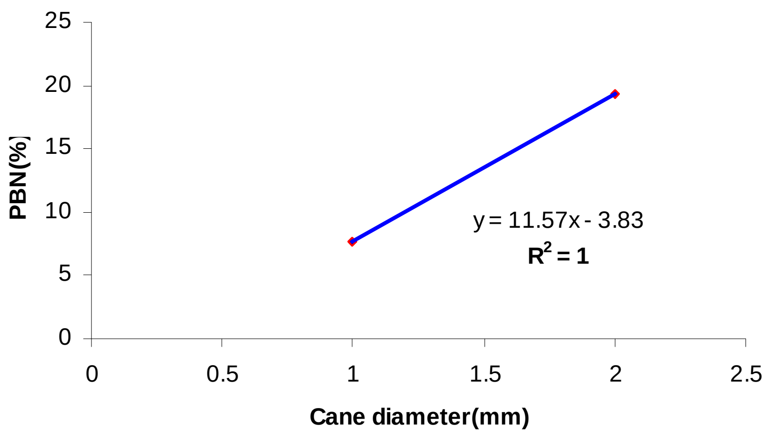


FIGURE 8 Correlation of primary bud necrosis (%PBN) with cane diameter in Askari grapevine.

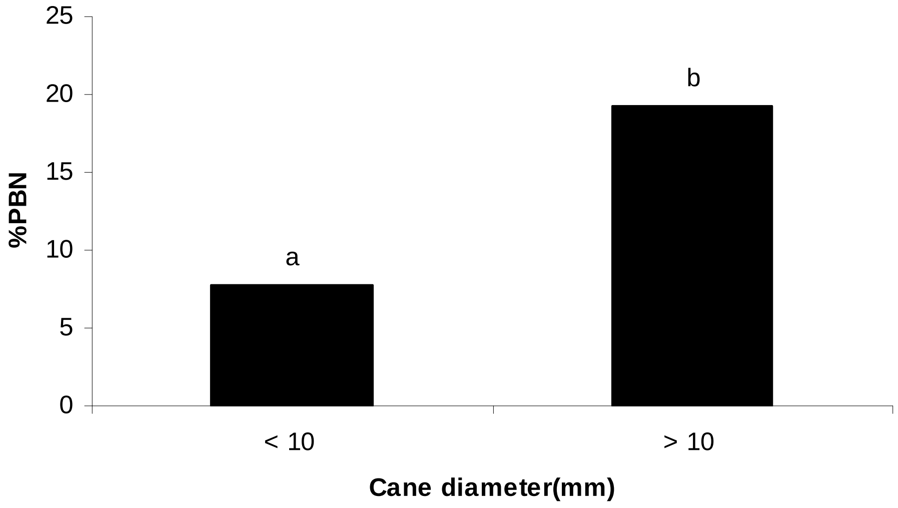


FIGURE 2 Effect of cane diameter on PBN% in Askari grapevine bud.
Values with the same letters in a histogram do not differ significantly (P≤0.01).

From a veiw point of the node position on canes, nodes 1 to 5 had a higher incidence of PBN (39.57%) wherase, lowest of PBN (0.2%) were observed in nodes 16 to 20(Fig. 3). So there was a negative correlation between bud position on cane and PBN percentage (Fig. 9).

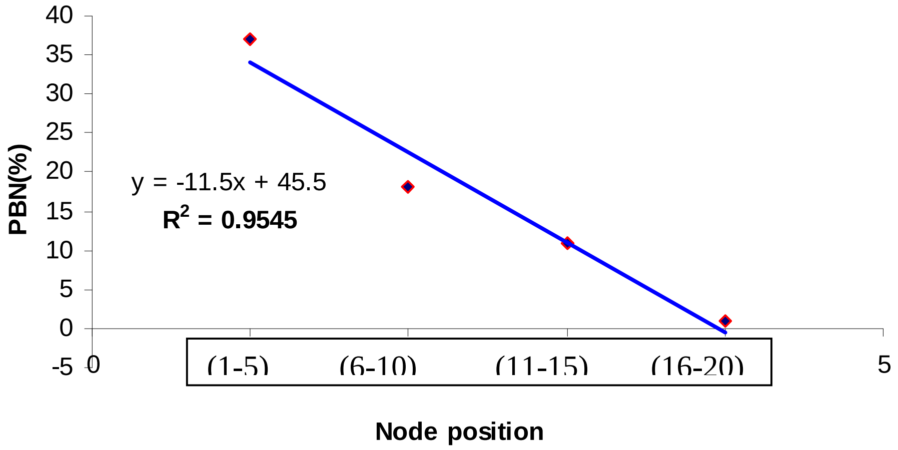


FIGURE 9 Correlation of primary bud necrosis (%PBN) with node position in Askari grapevine.

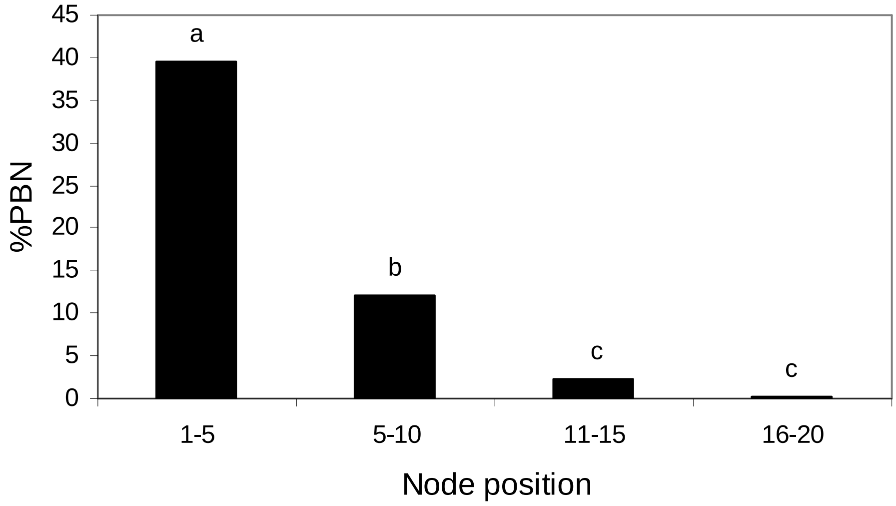


FIGURE 3 Effect of node position on PBN% in Askari grapevine.
Values with the same letters in a column do not differ significantly (P≤0.01).

The assessment of PBN showed that the highest incidence of PBN (18.09% and 18.78) was observed in February and March, respectively and the lowest incidence of PBN (6.28%) in November (the first sampling date) (Fig. 4). Consequently there is a strong correlation between sampling date and the incidence of PBN percentage in winter.(fig. 10).

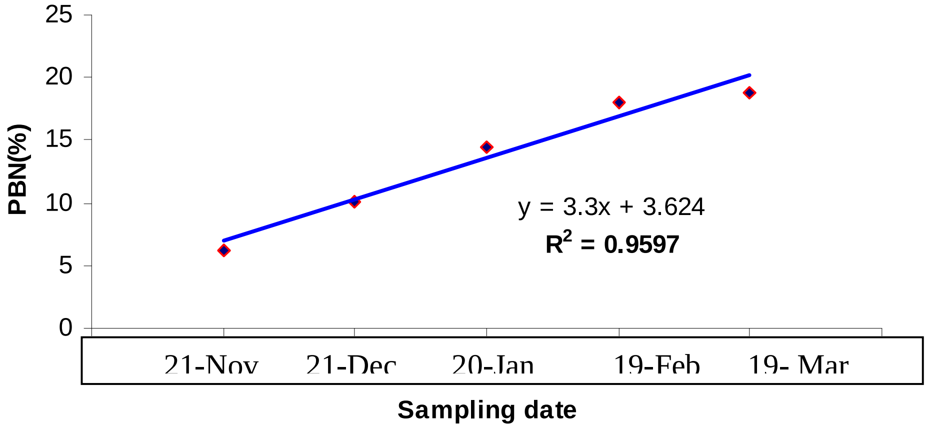


FIGURE 10 Correlation of primary bud necrosis (%PBN) with sampling date in Askari grapevine.

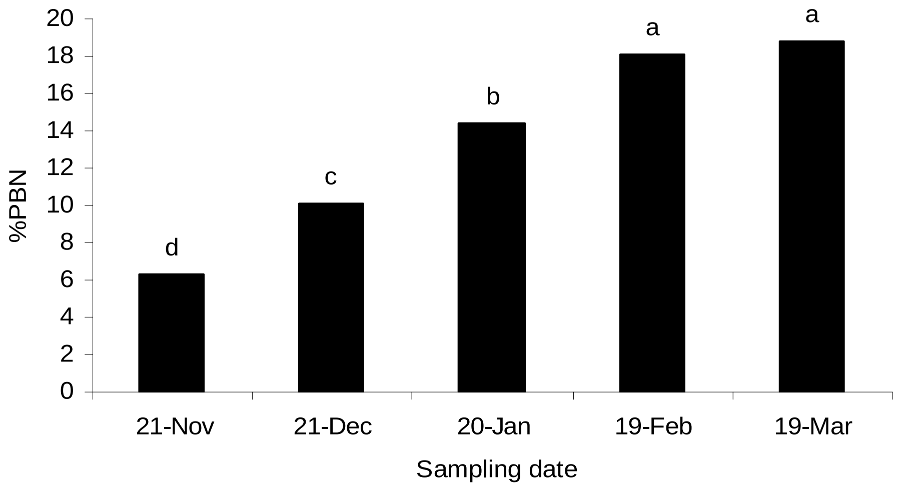
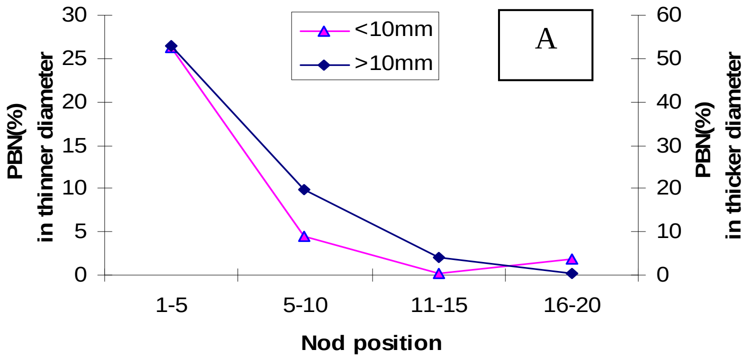
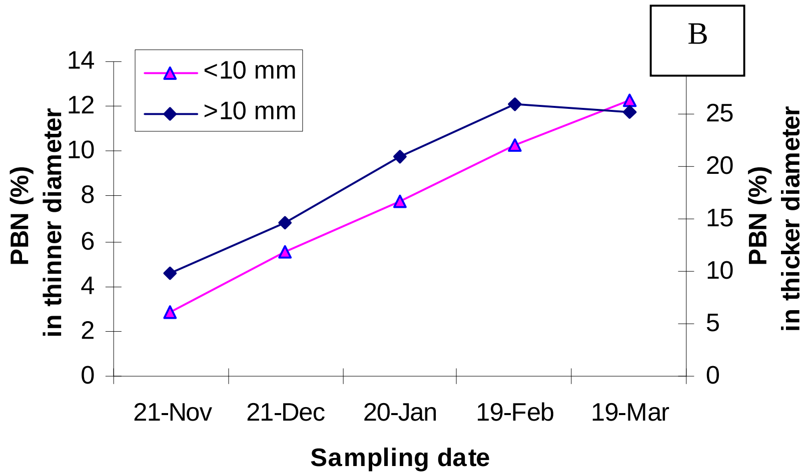


FIGURE 4 Effect of sampling date on PBN% in Askari grapevine.
Values with the same letters in a histogram do not differ significantly (P≤0.01).

There was a relationship between node position and the cane diameter, so that a higher and lower PBN percentage were obsereved in the basal and distal nodes, respectively. According to the obtained results, the highest incidence of PBN in both thick(> 10 mm) and thin(< 1o mm) canes were observed in proximal buds (nodes 1-5) and remarkably decreased in distal buds(nodes 11-20) (Fig. 5 A).

In both cane diameters (> 10 mm and < 10 mm), the lowest and highest PBN% were observed in November and March, respectively. Also in all sampling dates, the PBN incidence was highest in thicker canes as compared to thinner ones (Fig. 5 B). As sampling was delayed, the incidence of PBN was greater in lower bud compared to the higher position. In last sampling date (March) the percentage of PBN was 57.55% in nodes 1-5 (fig 5 C).





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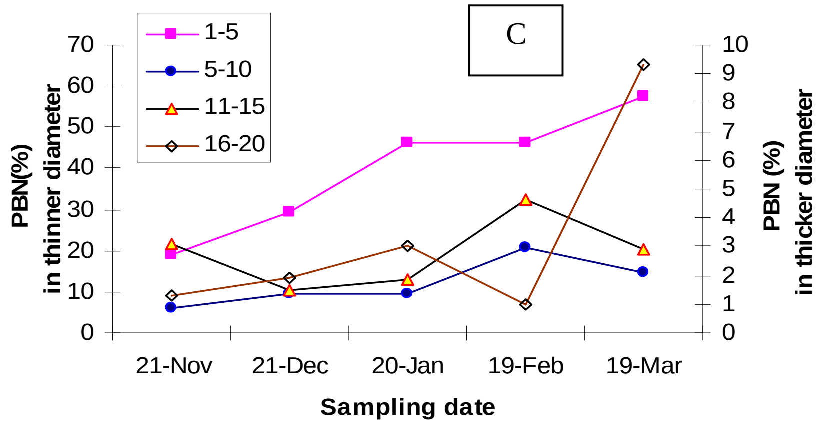

FIGURE 5 The relationship between PBN% and cane diameter, node position and sampling date in Askari grapevine.

Anatomical observations of Askari grapevine dormant buds, showed that PBN occurred in central buds as brown small spot and gradually developed in the whole bud and bud necrotic remained on canes and did not abscission. Necrotic buds was observed in Primary bud and sometimes the disorder occurred in secondary buds (Fig. 6).

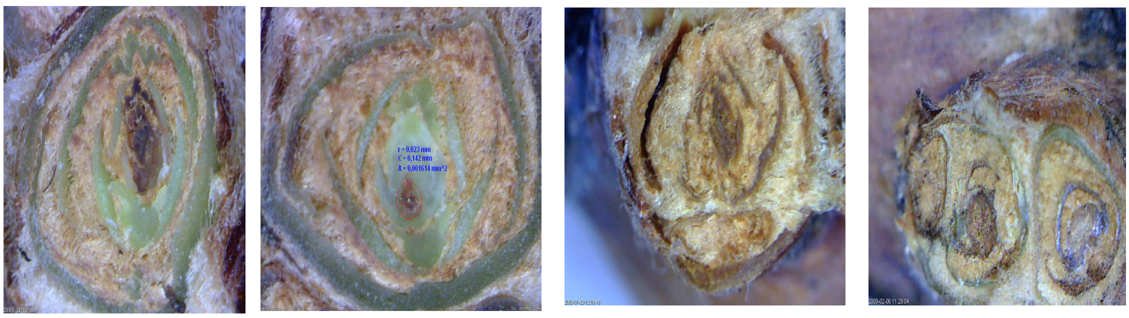

FIGURE 6 Cross section through a mature Askari grapevine dormant bud, Start of PBN (A), developing PBN (B), Full PBN (C) and primary and secondary and tertiary buds necrosis(D).

In healthy primary and secondary buds there was extensive damage, distorted tissue and finally case a splitting in PBN state. In most cases, tissue above of necrotic zone become completely brown and dry, while the tissue below that bud remained green color and health (Fig.7).

FIGURE 7 Cross sub section of both health (A) and death (B) primary bud with seperation tissues(C) in Askari grapevine dormant bud.

DISCUSSION AND CONCLUSION

The incidence of PBN is serius in Iran, and Askari is a cultivar which is one of the main table grapes grown in many areas is very suceptible to this physiological disorder of PBN. This study which is reported for the 1st time in Iran, clearly points out the importantance of this problem. The number of buds retained on the grapevine after pruning has a considerable impact on canopy extention and vine yield in the following season. If too few buds are retained due to PBN, yield may be reduced to below what the vine would otherwise have the capacity to ripen. Also, shoot grow may be excessively vigorous because of a lack of competition with the fruit and other shoots.

Our results clearly indicated that reduced yield in Askari cultivar at investigated vineyards directely related with the incidence of PBN. The results also showed that when primary buds are dead, secondary buds will growth which have a lower yield potential. The incidence of PBN on *Vitis vinifere* L. cultivar Askari in Southwest of Iran was identical to that described by Lavee *et al*. [12], Morrison and Iodi [13], Perez & kliewer [16] and Collins & Rawnsley [3]. In recent years, the use of bud dissection analysis has shown that some vineyards experience high levels of PBN which can ultimately reduce yield potential. Significantly high levels of PBN have also been observed in cultivars such as Cabernet Sauvignon, Riesling, Viognier and Chardonnay [7, 6, 17]. Determining the impact of PBN on a number of different red and white wine grape cultivars would be valuable to the wine industry, as most research overseas has been focused on table grapes such as Sultana, Flame Seedless, Thompson Seedless, Queen of the Vineyard and Kyoho [12, 14, 13, 7].

Vigorus growth, expressed as cane diameter, internode length and growth rate, has been associated with a high incidence of PBN. For example, 'Shiraz' cultivar is a highly vigorous cultivar and is prone to PBN. Our results concerning the relationship between cane vigor and incidence of PBN 'Askari' grapevine, is agreement with Dry & Coombe [6] and Lavee *et al*. [12] but was oposed to Naito *et al*. [15]. The correlation between vigour and the incidence of PBN may be associated with rapid shoot growth in spring. A rapid growth surge is related to increased levels of growth hormones causing abnormal tissue development. Dry [5] showed that the level of PBN is directly proportional to the severity of shoot topping, defoliation or shoot thinning. Severe shoot thinning increased the incidence of PBN in Shiraz, whereby removal of shoots promotes increased vigour of remaining shoots. However, studies in Chile showed that a modest level of shoot thinning reduced the incidence of bud necrosis in Sultana [16], while in Riesling the effect of shoot thinning on the incidence of PBN was variable between seasons [21].

There was a positive correlation between vigor and PBN incidence, and the difference between thick and thin canes from viewpoint of PBN incidence, was greatest at basal node, agreement to Lavee *et al.* [12] and Dry & coombe [6]. Also, yield reduction can be due to increasing the secondary to primary shoot ratio in vigorous vineyards. Previous reports indicated that PBN increased to the onset of bud dormancy [13, 12, 19]. Although our results supported this assumption, sampling throughout the entire season revealed the incidence of PBN could increase later. There is a probability that climatic and cultural conditions cause variability PBN incidence in vineyards.

Basal buds on the canes have a higher vigour potential and a lower reproductive differentiation rate, and these tend to develop a higher PBN incidence. Low fertility of basal buds is common in many cultivars in vigorous situation. There is many possible causes of PBN incidenc, that high shoot vigour [12, 6], canopy shading [16, 20], frost stress, low bud carbohydrate reserve, high GA3 level [22], high level of soil nitrogen [10], have all been shown to increase the incidence of PBN. Visually a bud with PBN appeas similar to that of a healthy bud and, therefore, difficult to detect by eye [6]. Although it is possible to see PBN in the field with a hand lens, bud dissection are need to accurately detect PBN and a assess bud fruitfulness. Microscopic bud dissection is being used to assess bud fertility and predict potential yield in vineyards. PBN disorder can be easily detected in dissected buds and also whether partial or complete necrosis has occurred. Similar to the observation by Vasudevan *et al*. [18], the first visible symptom of PBN was indicated by the presence of distorted and compressed cells with irregular cell walls. There were, however, some differences in the location of PBN. Vasudevan *et al*. [18] found that the zone of compressed cells began at the base of the primary bud and advanced to the leaf primordia. Our observations indicated that PBN start in the central zone of leaf primordia in some primary buds, not just at the base. Morrison & Iodi [13] also observed the random distribution of PBN in the early stages of development. In Thompson Seedless, PBN is characterised by the formation of a distinct necrotic zone most commonly located at the fourth leaf primordia [16]. Collins *et al*. [4] show that the location of cell breakdown due to PBN appeared to be random and was not isolated to one region within the primary bud.

The formation of necrotic cells in the primary bud caused a rupture or separation between the basal part of the bud and the apical meristem, resulting in death of the primary bud. PBN stopped further primordial growth, so that cells matured more rapidly without forming fully developed leaf primordia. This entire cell region of the primary bud then breaks down and, if severe, necrosis extended into the secondary buds. Cell seperation at the necrotic zone was due to cell breakage, rather than formation of an abscision zone. Results in this study showed that PBN were most affected and sometimes both of secondary buds showed necrotic symptom tha that this results was agreement with Bindra and Chohan [2].

It is concluded that primary bud necrosis is one of important factors of low fruitfulness in vineyards under consideration.. Also it was conclusion that PBN incidence and distribution of buds on thick and thin canes were correlated. However when buds along canes are detected prior to onset of spring, severe PBN can be observed. PBN disorder appears to be widespread throughout the Askari grapevine in some of vineyards under consideration. Also it is mentionable that distribution of PBN within canes was very variable, and there were large differences in the incidence of PBN between sampling dates, cane vigor and bud position. Many vineyards have showed a high incidence of bud necrosis, with up to 50% bud necrosis detected in some vineyards in areas such as the Sisakht, Southwest of Iran. In general, <20% PBN may not cause significant yield loss. Although it was expected that PBN levels would increase during the season, the incidence of PBN fluctuated. This verifies that, regardless of cultivar, bud fruitfulness must be assessed as close to pruning as possible to ensure pruning levels are modified accordingly. Therefore, fruitfulness can be estimated prior to pruning by dissecting of a sample of buds which allows a grower to optimize crop load by leaving the best combination of cane and spur length.

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