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The effect of bud-scale removal and gibberellin (GA_3) on dormancy break of apricot (*P. armeniaca* L.) vegetative buds

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Abstract

Effect of bud scale removal and different application rates of gibberellin (GA_3) on bud break of dormant vegetative buds of two apricot cultivars was investigated. Shoot explants of 'Jafari' and 'Rajabali' cultivars were collected from one-year-old dormant branches and cultured in woody plant medium (WPM), supplemented with 2 mg L^{-1} of benzyl amino purine (BAP), 0.04 mg L^{-1} 3-bndolebutyric acid (IBA). Treatments included three different concentrations of GA_3 (4, 6, and 8 mg L^{-1}), and removed and unremoved scale bud forms. The results indicated that none of the unremoved bud scales sprouted even when GA_3 treatments were used ($P \leq 0.01$). The average of bud sprouting in removed scales buds was 62.77 %. The maximum bud sprouting (72.5 %) occurred in treatment with 8 mg L^{-1} of GA_3 and removed bud scales. There was a significant difference between two apricot cultivars on vegetative dormant bud break percentage when scale removal of buds was done. The removed scale bud of 'Jafari' and 'Rajabali' cultivars sprouted 55.22 and 70.33 %, respectively. This study showed that the scales are probably containing inhibitory substances such as abscisic acid and by removing them, bud break will occur. Also, this method provides the possibility of *in vitro* culturing of apricot trees in non-growing seasons (winter).

Key words: Bud break, scale removal, growth inhibitors, plant growth regulator, tissue culture

Introduction

Bud dormancy is one of the most important physiological mechanisms, which consents most deciduous fruit tree cultivars to escape damage in inappropriate environments and conditions. During the dormant period, growth and development are suspended (Saure, 1985; Cooke *et al.,* 2012). Because of bud dormancy, fruit trees species synchronize their annual growth and development to surrounding climate zone. For next season vegetative growth and fruit production, deciduous fruit trees in the temperate zone need to an acquaintance by low temperatures to overcome endo-dormancy period (Saure, 1985; Cooke *et al.,* 2012). Different species and cultivars have a specific amount of required chilling hours for breaking bud dormancy. In areas with lack appropriate chilling requirement hours, deciduous fruit trees growers are using substances such as hydrogen cyananide, mineral oil, and potassium nitrate to persuade bud dormancy release (Saure, 1985; Sagredo *et al.,* 2005; Finkelstein *et al.,* 2008; Sabry *et al.,* 2011; Cooke *et al.,* 2012). At the end of each growing season, once the leaves have dropped off the branches, bud scales will be conFig.d. To develop both new leaves and the elongation of a shoot's growth bud scales configuration is needed by fruit trees. During the dormancy period, newly developing growth parts are surrounded by these flaking or pseudo-leaves, which protect them from the unsuitable elements until the tree is set to resume its new growth phase. In the spring shoots and leaves activate to grow again, once they become fully matured the bud scales expose and ultimately are shed by the tree (Swartz *et al.,* 1984). Bud scales perform as a physical protection for meristem, though it is uncertain if they have a role in the control of bud dormancy (Cooke *et al.,* 2012). However, there are some reports available indicating that bud-scale removal can replace chilling requirement and stimulate meristem growth in some species (Erez *et al.,* 1980). Swartz *et al.* (1984) have reported that bud scales removal enhanced bud dormancy break in apples. Inhibitory constituents of scales on bud break, and its role in dormancy and bud break of grapevine have been reported (Iwasaki, 1980; Mizutani *et al.,* 1985, 1995; El-Shereif *et al.,* 2006).

In deciduous fruit trees, hormones play important role in induction of dormancy, and gibberellins (GAs) have been reported to have a predominant role in bud break (Zhuang *et al.,* 2013). It has also been reported that GA is primarily important, and might have a role in the timing of dormancy formation and chilling-induced release (Schrader *et al.,* 2004). Applications of exogenous GA on some varieties of woody plants often persuade dormancy release. Saure (1985) also stated that GA application could replace chilling requirements toward dormancy release. It also proposed that bud burst is reliant on adequate of GA level, which also plays a key role in growth termination (Hoffman, 2011). Clemens *et al.* (1995) showed that application of GA_4 on vegetative buds of *Metrosideros collina* cv. Tahiti. nearby to expansion stage could stimulate bud break. Gibberellin (GA_4) promoted dormancy release in Japanese apricot (*Prunus mume* Sieb. Et Zucc) significantly when applied on flower buds (Zhuang *et al.,* 2013; 2015).

Because of dormancy break during winter, a tissue culture of deciduous fruit trees is limited to growing season. Also, there is little information available on the use of gibberellin in combination with bud scale removal to enhance bud break in apricot (*Prunus*

armeniaca L.). This study aimed to determine the influence of gibberellin with different concentrations accompanied with bud scale removal on vegetative buds of two apricot cultivars 'Jafari' and 'Rajabali' to promote bud dormancy release and develop an *in vitro* culture method to propagate apricot in winter during dormancy season.

Materials and methods

Plant materials: During winter (February), cuttings (10-15 cm) of one-year-old branches from mature trees 'Jafari' and 'Rajabali' apricots grown at research block at Shahrood University of Technology-Iran were collected.

Scale removing and explant preparation: Cuttings were transported to the lab and bud scale removal was carefully done using a sterile scalpel blade. Before applying the treatments, cuttings were washed with tap water, sodium hypochlorite (1 %), and rinsed finally with deionized water. To disinfect the explants, they were placed in mercuric chloride ($HgCl_2$, 1000 ppm) for 4 min, and then 3 min in citric acid solution (7000 ppm). To finish the disinfection, they were washed three times with deionized water. Explants disinfection was carried out under complete aseptic conditions under the laminar airflow hood.

Tissue culture: The disinfected explants was transferred to woody plant media culture (WPM, McCown and Lloyd, 1981), which included sucrose 3 %, agar 8 g L^{-1}, active charcoal 4 g L^{-1}, BAP 2 mg L^{-1}, IBA 0.04 mg L^{-1}, and gibberellin (GA_3) in different concentrations (4, 6, 8 mg L^{-1}). The media was autoclaved for 20 min at 121 °C and 1.1 kg/cm^2 pressures for sterilization, then left to cool to the ambient temperature. Explants were incubated in a growth chamber with 16 hours of light and 8 hours of darkness at 25 °C. All materials used in this study was purchased from Merck (KGaA, Germany)

Statistical analysis: The experiment was laid out as a completely randomized design (CRD) with a factorial combination of treatments and three replications of each treatment combination. Treatments were the two apricot cultivars ('Jafari' and 'Rajabali'), removed and unremoved bud scales, and different application rate of GA_3 (4, 6, and 8 mg L^{-1}). Data were analyzed by analysis of variance (ANOVA), mean treatment differences among the treatments were tested at $P \leq 0.05$, by Duncan's multiple range test. SAS, version 9.3 statistical software (SAS Institute, Cary, NC, USA) was used for data analyzing.

Results

The ANOVA results showed that there were significant differences ($P \leq 0.01$) among the treatments; scale removal, GA_3, cultivar, scale × GA_3, and scale × cultivar on bud break percentage (Table 1). The average bud break percentage for removed bud scales treated with GA_3 was 62.77 %, while no bud break was observed for the unremoved bud scales treated with different application rates of GA_3 (Fig. 1, 2). As shown in Fig. 3, the highest and the lowest bud break percentage were recorded for GA_3 8 mg L^{-1} and GA_3 4 mg L^{-1} treatments, respectively. A significant difference ($P \leq 0.01$) was observed between apricot cultivars for the bud break percentage (Fig. 4). The bud break percentage was higher for 'Jafari' (35.16 %) cultivar than 'Rajabali' cultivar (27.61 %).

The effect of GA_3 application rate × scale removal on bud break

Fig. 1. Growing buds in removed scale buds of apricot cv. Jafari after two week of culture on WPM.

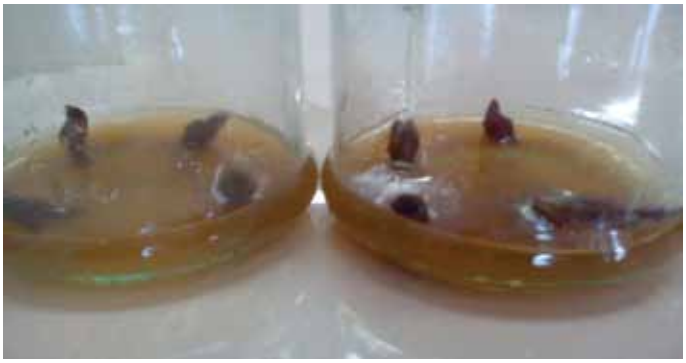

Fig. 2. Lack of bud break in unremoved scale buds of apricot cv. Jafari after two week of culture on WPM

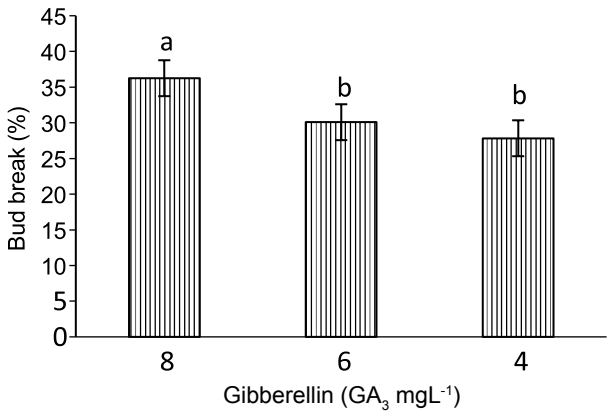

Fig. 3. Effect of different gibberellin (GA_3) concentrations on bud break percentage. Means indicated with different lowercase letters are significantly different at $P < 0.01$ according to Duncan's Multiple Range Test.

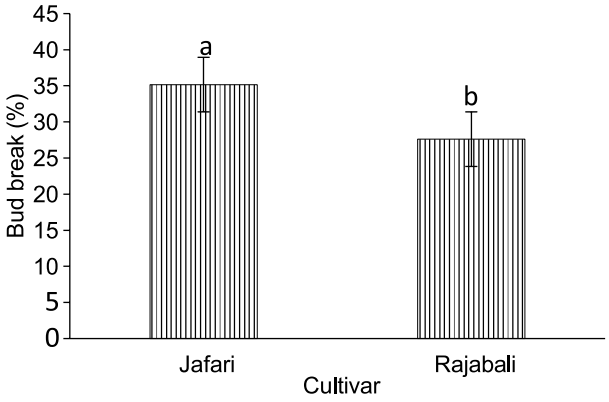

Fig. 4. Responding apricot cultivars to bud break percentage. Means indicated with different lowercase letters are significantly different at $P< 0.01$ according to Duncan's Multiple Range Test

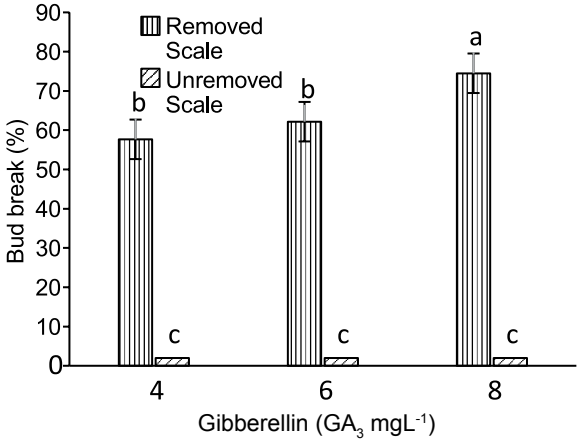

Fig. 5. Effect of different gibberellin concentrations (GA3) and scale removal on bud break percentage. Means indicated with different lowercase letters are significantly different at $P< 0.01$ according to Duncan's Multiple Range Test

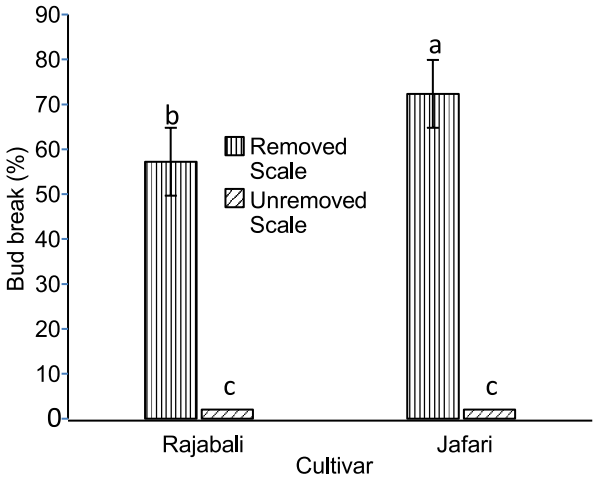

Fig. 6. Effect of scale removal on bud break percentage in both apricot cultivars. Means indicated with different lowercase letters are significantly different at $P< 0.01$ according to Duncan's Multiple Range Test

percentage was significant at $P\leq 0.01$ (Table 1). The removed bud scales showed the highest germination percentage (72.5 %) on WPM at GA_3 application rate of 8 mg L^{-1}, while the lowest bud germination percentage (55.66 %) was observed at a GA_3 application rate of 4 mg L^{-1} (Fig. 5). However, the difference between application rate of 4 mg L^{-1} and 6 mg L^{-1} was not significant. According to Table 1 and Fig. 6, bud break percentage

Table 1. The effect of gibberellin (GA_3) and scale removal on breaking dormancy of vegetative buds in two apricot cultivars ('Jafari' and 'Rajabali')

Treatment	DF	Bud break (%)
Removed scale	1	35469.44**
Gibberellin	2	227.86**
Cultivar	1	513.78**
Removed scale × Gibberellin	2	227.86**
Removed scale × Cultivar	1	513.78**
Cultivar × Gibberellin	2	54.53^{ns}
Removed scale × Cultivar × Gibberellin	2	54.53^{ns}
Error	24	33.75
CV %	-	18.50

** significant differences at $P \leq 0.01$.

^{ns} no significant differences at $P \leq 0.01$.

was significantly affected ($P \leq 0.01$) by cultivar and scale removal interaction. In the removed bud scales, the germination rate on the WPM for both 'Jafari' and 'Rajabali' cultivars were 70.33 and 55.22, respectively (Fig. 6).

Discussion

In vitro culture of most deciduous fruit trees is limited to growing season (spring and summer). During autumn and winter, deciduous trees respond to symptoms such as cold weather and day length and move to dormant phase. Dormant buds do not have the vigor same as buds during the growing season (Saure, 1985; Cooke *et al.,* 2012). The results showed (Fig. 5, 6) that the scale removal help to break bud dormancy in apricot trees in the *in vitro* culture conditions. There are some evidences showing that during dormancy period, inhibitory elements accumulate in the bud scales and prevent the regrowth (Wun *et al.,* 2007). Abscisic acid is one the most important inhibitor that causes bud dormancy in the plant. This compound was reported to delay the flowering buds break in some apple cultivars effectively when the bud was cultured in *in vitro* condition (Dutcher and Powell, 1972; Singha and Powell, 1978). A negative relationship between increasing amount of abscisic acid with bud break in apple, cherry, peach, and grape have been reported (Mielke and Dennis, 1978; Zheng *et al.,* 2015). It seems by eliminating the abscisic acid in the dormant buds germination vigor can turn back to the explants. Observation of this study indicate that the presence of abscisic acid as a growth inhibitor preventing bud break and germination in unremoved bud scales of apricot cultivars. Some researchers observed that in the unremoved bud scales even with the help of GA treatment germination did not occur in any of the explants (Wun *et al.,* 2007). Research on apples grown in subtropical regions indicated that defoliation three weeks after harvesting triggered buds break immediately without having dormancy period (Janick, 1974; Notodimedjo *et al.,* 1981). This study also confirmed that the bud break inhibitors are most likely produced in leaves and transmitted to the bud scales before falling leaves. If the inhibitor(s) transfer before falling leaves, bud break will not happen (Wun *et al.,* 2007). Changes in endogenous levels of abscisic acid in the bud from the dormancy beginning to the dormancy breaking (during cold weather period) in many species have been reported (Powell, 1987: Rodriguez *et al.,* 1991; Zheng

et al., 2015). Thus, when bud break occur, the level of abscisic acid is in the lowest amount (Dennis, 1961). Some reports have suggested that abscisic acid level starts increasing in autumn and reaches to its maximum level by early winter, and then decreases to the point of breaking dormancy (Lang, 1989; Powell, 1987; Wun *et al.,* 2007). It is possible that the level of abscisic acid decreased by 62.77 % in apricot buds using scale removal, where the bud break percentage was observed 33.16 and 27.61 for cultivars 'Jafari' and 'Rajabali', respectively. Germination of removed bud scales have been reported as a sign of abscisic acid elimination by removing scale from buds (Powell, 1987; Swartz, 1984; Wun *et al.,* 2007).

In the current study, the studied apricot cultivars reacted differently to the scale removal treatment, which indicates the response to the bud break is a cultivar depended variable (Fig. 4). The variation for the chilling unit requirement for reproductive buds break dormancy in apricot cultivars was reported considerably different (Wun *et al.,* 2007). Scale removing was reported more effective on bud break in late season apple than the early one (Swartz *et al.,* 1984). The response rate to bud break was recorded 70.33 and 55.22 % for 'Jafari' and 'Rajabali' cultivars, respectively (Fig. 6). Bud dormancy in fruit tree production is important for managing orchard, however available information in this field is less than information available for seed dormancy. Furthermore, most research works in fruit tree were done on dormancy in reproductive buds, and there is little information available for the vegetative buds. Temperature, light, humidity, and nutrient are also important factors on bud dormancy (Powell, 1987; Wun *et al.,* 2007). In this study, the apricot dormant vegetative buds were transferred from the field to the lab, after applying the treatments on them they were cultured on the WPM and transferred to a growth chamber at 25 ˚C, 16 hours light, and 8 hours dark. Also, the *in vitro* condition used in this study might have effects on apricot buds break.

The results showed that GA_3 increased bud break percentage (72.5 %) in apricot vegetative buds. The greatest effect of GA_3 on the bud break percentage of apricot was observed at the application rate of 8 mg L^{-1} using removed bud scales (Fig. 5). Zhuang *et al.* (2013, 2105) showed that GA_4 treatment induced earlier bud break in Japanese apricot reproductive buds. More research has been done on effects of GAs on seed germination (seed dormancy break) than their applications on fruit vegetative buds (Wun *et al.,* 2007; Finkelstein *et al.,* 2008; Zhuang *et al.,* 2013, 2105). One of the positive effects of chemical stimulators such as gibberellic acid on seed germination is likely to achieve the balance of hormones in seed and reduces the growth inhibitors effect such as abscisic acid (Finkelstein *et al.,* 2008). As a result, gibberellic acid biosynthesis through the induction of alpha-amylase enzyme that triggers seed dormancy break (Finkelstein *et al.,* 2008; Gupta and Chakrabarty, 2013; Zheng *et al.,* 2015). To evaluate endogenous changes of GAs level in the bud, research has also shown that an increase in the GAs level caused the dormancy break in buds (Little and Macdonald, 2003; Wun *et al.,* 2007). As noted in the materials and methods part of the current study, a small amount of cytokinin (BAP 2 mg L^{-1}) was used in tissue culture medium. The external effects of cytokinin on growth and bud dormancy are not yet known. Though, some research has shown that dormancy can break with external use of cytokinin. However it alone had little effect on breaking dormancy and cannot replace cold (Hartmann *et al.,* 1990; Wun *et al.,* 2007; Gupta and Chakrabarty, 2013). Although, the ratio of growth regulator such as GAs and cytokinin to abscisic acid is more important than their concentration to break the dormancy in the bud (Wun *et al.,* 2007; Gupta and Chakrabarty, 2013).

This study suggests that growth inhibitors such as abscisic acid may exist in the apricot bud scales. Reducing abscisic acid as an inhibitor by scale removal and increasing the level of growth hormones such as cytokinin and gibberellin at the same time lead to a hormonal imbalance that triggers the bud break dormancy. Also, using plant tissue culture techniques and applying appropriate hormonal treatments the sprouting buds can be transferred to proliferation medium, and then a rooting medium,.This method can be used to propagate deciduous fruit trees during dormancy period in autumn and winter, as we observed in this study.

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