

Optimization of plant growth regulators for *in vitro* shoot proliferation of apple cv. 'Abbasi' using response surface method

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Abstract

Optimization of medium components in *in vitro* cultures is very difficult because of having multifactor nature. To optimize the plant growth regulator concentrations (BA, IBA, GA₃) for shoot proliferation of apple cv. 'Abbasi' a three-factor experiment was designed and performed in 20 points (20 treatments) using Design Expert software based on the response surface method. A completely randomized design using eight auxin combinations (IBA+NAA) was also carried out for *in vitro* rooting of microcuttings. Proliferated shoot number was linearly and positively affected by the BA factor, while the response of the leaf number was linearly and negatively affected by BA. The effect of GA₃ on proliferated shoot length was negative and linear. The abnormal shoot percentage in a quadratic model was influenced by all three types of PGRs, i.e. BA, IBA and GA₃; positively and linear by BA, positively and quadratic by IBA and negatively and quadratic by GA₃. According to the results of this experiment, the optimum concentrations for BA, IBA and GA₃ combination were 1.5, 0.1 and 0.02 mg L⁻¹, respectively, which resulted in the proliferated shoots with a rate of 2.5 shoots per explant, with 0.9 cm in length, 4.3 leaves per shoot and 4% abnormal shoots. The percentage of shoot-tip necrosis was not affected by the evaluated factors. NAA was more effective than IBA for rooting microcuttings. The medium supplemented by 1 mg L⁻¹ IBA or 0.5 mg L⁻¹ NAA can be used successfully for rooting of apple cv. 'Abbasi' shoots *in vitro*.

Keywords: BA; Design Expert software; GA₃; IBA; *In vitro* propagation; *Malus domestica* Borkh.

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Introduction

Micropropagation of apple has an important role in the rapid propagation of cultivars and rootstocks with favorable properties and it is a part of the breeding studies (Korban and Chen 1992; Ciccoti *et al.* 2008; Hemmaty *et al.* 2014; Jalali Samghabadi and Jalili Marandi 2015) and production of healthy (disease-free) plants. (O'Herlihy *et al.* 2003; Paprstein *et al.* 2008). The success and failure of micropropagation protocols depend on the proliferated shoot rate. It is affected

by several factors under *in vitro* conditions such as genotype, medium composition, environmental factors, and so on (Dobrąnszki and Teixeira da Silva 2010). Proliferated shoot rate is dependent on the initiation and activation of lateral meristems controlled by hormones, mainly cytokines; however, cytokines interact with auxin, even though the auxin effect is indirect (Ward and Leyser 2004). Regenerated shoot production of apples in the medium containing cytokinin, as the

main growth regulator, and low concentrations of auxin and in some cases GA₃ has been reported (Yepes and Aldwinckle 1994; Kaushal *et al.* 2005). The effect of different growth regulators on the proliferation stage is strongly dependent on the genotype (Lane and McDougald 1982; Welander 1985; Marin *et al.* 1993). In many studies on apple shoot proliferation, BA has been used as a source of cytokinin and mainly in the range between 0.5 and 1 mg L⁻¹. Golden Delicious cultivar led to the maximum proliferated shoots when BA at 1 mg L⁻¹ was used (Baraldi *et al.* 1991). Marin *et al.* (1993) obtained a high rate of shoot proliferation when using 1 or 2 and 2 mg L⁻¹ of BA for Greensleeves and Tuscan (SC:7-10) cultivars, respectively. Yepes and Aldwinckle (1994) reported that BA at 1 mg L⁻¹ is the best concentration for some apple cultivars and rootstocks. The concentration of 0.5 mg L⁻¹ of BA, 1 and 1 mg L⁻¹ of BAR was used for optimum shoot proliferation in M.26, MM.106 and JTE-H rootstocks, respectively (Dobranszki *et al.* 2000a). MM.106 was well proliferated at 0.5 mg L⁻¹ of BA (Sharma *et al.* 2000). The dose of 1 mg L⁻¹ BA was also a suitable concentration for the shoot proliferation of MM.111 (Kaushal *et al.* 2005). The effect of IBA concentrations on apple shoot length was only observed in the presence of GA₃ (Yepes and Aldwinckle 1994), whereas the best shoot proliferation was observed in the media supplemented with BA+GA₃ and without auxin (Kaushal *et al.* 2005). The effect of different cytokinin derivatives (Dobranszki *et al.* 2000a; Magyar-Tabori *et al.* 2001a, b) and the simultaneous application of two types of cytokines (BA, BAR, TOP, KIN) (Modgil *et al.* 1999; Dobranszki *et al.* 2000b, c; Kaushal *et al.* 2005) has

also been studied for apple shoot proliferation and BA or BAR at 0.5 or 1 mg L⁻¹, KIN at 1 or 1.5 mg L⁻¹ and TOP at 3.5 or 5 mg L⁻¹ were effective.

It is important to use modeling systems to determine the optimum levels of the investigated factors due to the difficulty of optimizing the medium growth regulators. As multifactor studies have been successfully applied in numerous experiments to optimize the medium composition, the Design Expert software can be used to predict optimal factors of the medium (Niedz and Evens 2006, 2007; Niedz *et al.* 2007; Gago *et al.* 2010; Gallego *et al.* 2011; Karimpour *et al.* 2020). Response surface methodology (RSM) allows a geometric dimension to be assigned to each factor, then the created geometric volume (N dimension) is the experimental design space. Several points in the experimental design space are randomly assigned to specified sampling and formulations according to the responses obtained from each of these points, so the multifactorial tests provide more information than the single-factor tests (Niedz and Evens 2007).

In addition to the shoot proliferation rate, rooting is also an important stage in micropropagation. The type of auxin and its optimum concentration for rooting depends on the genotype. M.26, MM.106 and JTE-H rootstocks were rooted in 1, 2 and 1 mg L⁻¹ of IBA, respectively (Magyar-Tabori *et al.* 2002) while the MM.106 rootstock and the Gale Gala cultivars rooted at 3 and 1.5 mg L⁻¹ IAA, respectively (Sharma *et al.* 2007). IBA and NAA are used for rooting in apple cultivars and rootstocks, and IBA has been reported to be more effective than NAA for most apple cultivars, although in some cultivars

no significant difference was observed between the rooting ability of auxin types (Lane and McDougald 1982; Welander 1985; Zimmerman and Fordham 1985; Yepes and Aldwinckle 1994; Sharma *et al.* 2000; Ghanbari 2014).

This experiment was aimed to use the RSM method to find the optimum level of BA (Merck Co.), IBA (Merck Co.) and GA₃ (Merck Co.) for shoot proliferation of the apple cultivar 'Abbasi' in the MS medium, as well as to demonstrate the possibility of using advanced statistical software to improve the experimental design for optimizing the *in vitro* growth. Also, rooting of apple microcuttings using IBA and NAA combination treatments was evaluated.

Materials and Methods

Experiment 1: Shoot Proliferation

Plant Materials: Vegetative buds of the apple cultivar 'Abbasi' (*Malus domestica* Borkh. cv. 'Abbasi') from current season shoots of apple trees in the Shahroud Agricultural Research Center and Natural Resources, Iran, were collected and then, were transferred to the Tissue Culture Laboratory of Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. After washing with tap water, the buds were disinfected with ethanol 70% (1 min), and then with sodium hypochlorite solution 2% (20 min). Single node explants were cultivated in the MS medium (Murashige and Skoog 1962), supplemented by 1 mg L⁻¹ BA and 0.05 mg L⁻¹ IBA, 30 g L⁻¹ sucrose and 8 g L⁻¹ agar. After one subculture in the same medium, the uniform and identical established shoots (3 cm length) were used for the shoot proliferation experiment.

Statistical Design and Analysis: A three-dimensional design space defined by the plant growth regulators (BA, IBA, GA₃) for the shoot proliferation stage was created at the range of concentrations recommended in previous studies (Dobranszki and Teixeira da Silva 2010). Then, growth characteristics of shoots were evaluated in the designed treatments inside or on the 3D space surface. A predicted equation was created for each response. The concentration ranges of the three growth regulators were 0.5 - 2.5 mg L⁻¹ for BA and 0 - 0.5 mg L⁻¹ for IBA and GA₃. Twenty points were designed for the treatments (Figure 1). All 20 designed points were run simultaneously. For each treatment, 10 replicates (each replicate had one shoot in a 90 ml jar containing 10 ml medium) were assayed and were recorded after two subcultures (every four weeks in the same medium).

Number of proliferated shoots (number of regenerated shoots per explant), length of regenerated shoots (cm), number of leaves per shoot, shoot-tip necrosis (%) and abnormal shoots (%) were recorded. Abnormal shoots were recorded according to the deformity percentage from 0 to 100%.

Responses for each point were averaged over 10 replicates. For each response, the highest-order polynomial model that was significant at the 5% level was analyzed using analysis of variance (ANOVA) (Niedz and Evens 2006, 2007; Niedz *et al.* 2007). Design Expert® 8 (2010) software was used for designing, model evaluation and analysis.

Experiment 2: Rooting

Plant Material: *In vitro* shoots of apple cultivar

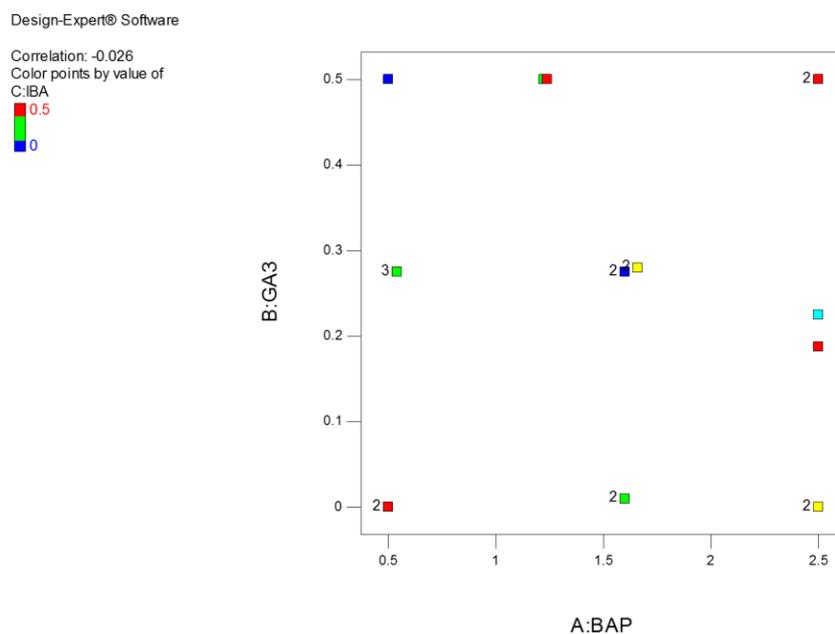


Figure 1. Points were designed by Design-Expert software for three-factor BA, IBA and GA₃. Numbers in each point indicate replication in a point. The unit for each factor is mg l⁻¹ (BAP: 0.5 – 2.5 mg l⁻¹, GA₃ and IBA: 0-0.5 mg l⁻¹).

‘Abbasi’ (3 cm) grown in the MS medium containing 1 mg L⁻¹ BA were used for this experiment. Table 1 shows the rooting treatments containing different combinations of IBA and NAA. The shoots were transferred to the rooting medium (MS, 20 mg L⁻¹ sucrose, 8 g L⁻¹ agar) based on the rooting treatments. The rooted shoots were transferred to small pots filled with the perlite: coco-peat (2:1) mixture. Plantlets were fed with ¼MS (one month), ½MS (two months) and then MS, respectively.

Statistical Design and Analysis: Rooting percentage was recorded 30 days after the beginning of the period. The experiment was conducted as a completely randomized design with five replications (10 micro-cuttings in each replicate). SAS software (SAS 1989) was used for

the statistical analyses and the means were compared using Duncan’s multiple range test at the 5% level of probability.

Cultures of two experiments were kept in the controlled growth chamber at 24±2 °C, 16/8 hrs. light /darkness; a light intensity of 40 μM m⁻¹ s⁻¹ was provided by white fluorescent lamps.

Results and Discussion

Experiment 1: Shoot Proliferation

Evaluated factors (BA, IBA, GA₃) had significant effects on the recorded responses in the statistical models (linear and quadratic polynomial) according to ANOVA. Shoot-tip necrosis was not affected by any of the factors (Table 2), indicating that apple shoot-tip necrosis can be influenced by other environmental factors such as mineral compounds and solidifying agent (Pasqualetto *et*

al. 1988; Singha *et al.* 1990; Grigoriadou *et al.* 2000; Bairu *et al.* 2009; Reed *et al.* 2013a). Similarly, Thakur and Kanwara (2011) reported that shoot-tip necrosis varied in different pear genotypes and was not affected by different concentrations of BA and IBA.

Proliferated Shoot Number

The number of regenerated shoots was affected by BA factor ($p \leq 0.0004$) in a linear model (Table 2) so that the number of regenerated shoots increased with the increment of BA concentration. Increasing BA up to 2.5 mg L^{-1} increased shoot production to 4.39 shoots per explant (Figure 2). Other factors had no significant effect on the number of regenerated shoots (Table 2). Lane and McDougald (1982) also found lower shoot number at the low concentration of BA ($1 \text{ } \mu\text{M}$) for different apple cultivars (1.22 for ‘M.27’, 0.5 for ‘M.9’ and ‘M.26’, and 1.47 for ‘Macspur’) as compared with the higher concentrations (up to $10 \text{ } \mu\text{M}$). On the other hand, $10 \text{ } \mu\text{M}$ concentrations of BA decreased the number of shoots in ‘M.26’ (2.72) and ‘Macspur’ (3.10) but this decrease was less than when used at low concentration (1 mM). The optimum concentration of BA (5 and $10 \text{ } \mu\text{M}$) to produce the maximum number of shoots (3.14-7.54 shoots per explant) were depended on the type of cultivars. A similar effect of increasing BA concentration (1.1 - $15.4 \text{ } \mu\text{M}$) on shoot number was also reported in ‘Akeró’ (Welander 1985) as the optimum BA concentration for shoot regeneration was about $8.8 \text{ } \mu\text{M}$ and at higher concentrations, no increase in the number of shoots was observed. However, the amount of shoot vitrification showed no increase. In contrast, the best shoot regeneration

(4-5 shoots per sample) and the best growth of MM.111 were observed in the medium with 0.5 - 1 mg L^{-1} BA and 0.5 mg L^{-1} GA₃ without auxin (Kaushal *et al.* 2005).

Proliferated Shoot Length

Proliferated shoot length was affected by GA₃ ($p \leq 0.01$) in a linear model (Table 2) so that with increasing GA₃ concentration, the proliferated shoot length decreased. Other factors had no significant effect on this character. GA₃ elimination led to the shoot length increase of up to 0.94 cm , while application of 0.5 mg L^{-1} GA₃ produced shoots with 0.31-cm length (Figure 3). It has been reported that the addition of GA₃ (0.5 mg L^{-1}) to medium containing BA (1 mg L^{-1}) and IBA (0.05 mg L^{-1}) reduced shoot length of ‘Merton I. 79’ rootstock (Soni *et al.* 2011). On the other hand, the results of this experiment were not in agreement with the studies reporting the positive effect of GA₃ on shoot length improvement. Yepes and Aldwinckle (1994) found that at a constant concentration of BA (1 mg L^{-1}), the balance between GA₃ and IBA affected the shoot length of some apple cultivars, thus, when the IBA level changed from 0.1 to 1 mg L^{-1} the shoot length increased but only in the presence of GA₃ (0.5 mg L^{-1}). At optimum BA concentrations, shoot growth of *Malus × domestica* cv. G.30 was affected by GA₃, whereas it was unaffected at lower concentrations of BA (Geng *et al.* 2016).

Leaf Number

Leaf number was affected by the BA factor ($p \leq 0.0014$) in a linear model (Table 2) so that with increasing BA concentration, leaf number

Table 1. Auxin combination treatments for *in vitro* rooting of micro-shoots in *Malus domestica* cv. 'Abbasi'

| Treatment | IBA (mg L ⁻¹) | NAA (mg L ⁻¹) |
|-----------|---------------------------|---------------------------|
| 1 | 0 | 0.5 |
| 2 | 0.5 | 0 |
| 3 | 0.5 | 0.5 |
| 4 | 1 | 0 |
| 5 | 0 | 1 |
| 6 | 2.4 | 0 |
| 7 | 3.3 | 0 |
| 8 | 1.2 | 0.3 |

Table 2. Summary of statistical analyses and model output formula for five shoot proliferation responses of *Malus domestica* cv. 'Abbasi' shoots *in vitro* affected by three PGR factors (BA, IBA, GA₃).

| Response | p-value | | | Formula | R-Squared | Adj R-Squared | Pred R-Squared |
|--|---------|---|-------------|--|-----------|---------------|----------------|
| | Model | Factors | Lack of fit | | | | |
| Proliferated Shoot Number (Linear Model) | 0.0004 | BA: 0.0004 | 0.742 | Y = 1.569 + 0.5 BA | 0.57 | 0.54 | 0.44 |
| Proliferated Shoot Length (Linear Model) | 0.0104 | GA ₃ : 0.0104 | 0.738 | Y = 0.729 - 0.21 GA ₃ | 0.33 | 0.29 | 0.19 |
| Leaf Number (Linear Model) | 0.0014 | BA: 0.0014 | 0.538 | Y = 1.51 - 0.47 BA | 0.44 | 0.41 | 0.30 |
| Shoot-tip Necrosis | ns | - | - | - | - | - | - |
| Abnormal Shoot (Quadratic Model) | 0.0032 | BA: 0.0043 IBA ² : 0.0082 GA ₃ ² : 0.045 | 0.344 | Y = -1.43 + 3.01 BA + 4.69 IBA ² - 3.14 GA ₃ | 0.59 | 0.55 | 0.44 |

ns: non-significant

Adj R-Squared: Adjusted R-Squared

Pred R-Squared: Predicted R-Squared

decreased. Other factors had no significant effect on the leaf number. The highest number of leaf production (7.9 per shoot) was obtained when BA was used at 0.5 mg L⁻¹ and the number of leaves decreased to three leaves per shoot with an increasing BA concentration of up to 2.5 mg L⁻¹ (Figure 4). It seems that decreasing the BA concentrations has led to higher leaf production by increasing the number of regenerated shoots and focusing on the shoot growth.

Abnormal Shoots

The percentage of abnormal shoots was affected by BA ($p \leq 0.0043$), IBA ($p \leq 0.0082$) and GA₃ ($p \leq 0.045$) in a quadratic polynomial model (Table 2). As shown in Figure 5 (a and b), lower concentrations of BA were effective in controlling the abnormal shoot production, and both IBA and GA₃ had a significant effect on the percentage of abnormal shoots at the optimum concentration of 0.25 mg L⁻¹ for each of them. Marin *et al.* (1993) reported that high BA concentrations led to short

Design-Expert® Software
 Factor Coding: Actual
 Original Scale
 Proliferated Shoot Number
 --- 95% CI Bands
 X1 = A: BA
 Actual Factors
 B: GA3 = 0.25
 C: IBA = 0.25

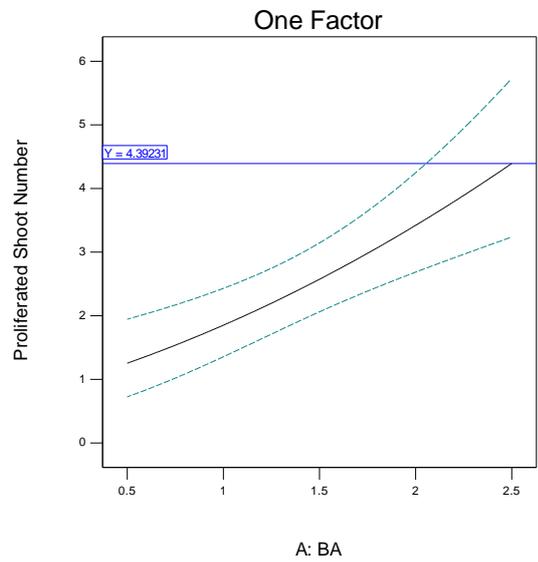


Figure 2. Proliferated shoot number of *Malus domestica* cv. 'Abbasi' affected by BA factor *in vitro*.

Design-Expert® Software
 Factor Coding: Actual
 Original Scale
 Proliferated Shoot Length (cm)
 --- 95% CI Bands
 X1 = B: GA3
 Actual Factors
 A: BA = 1.5
 C: IBA = 0.25

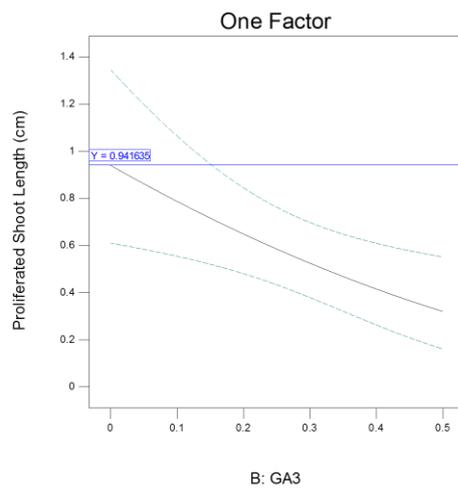


Figure 3. Proliferated shoot length of *Malus domestica* cv. 'Abbasi' affected by GA₃ factor *in vitro*.

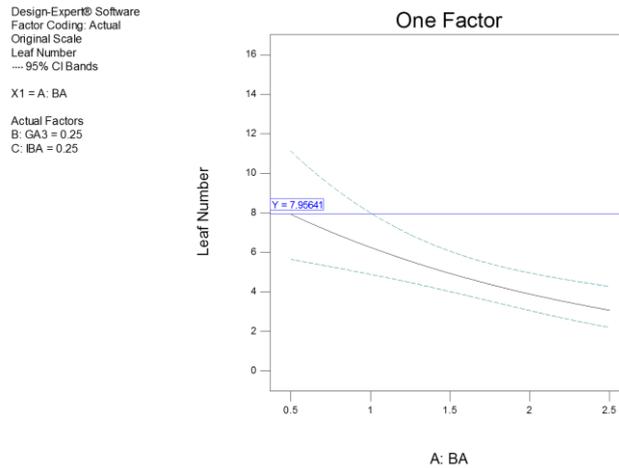


Figure 4. Leaf number of *Malus domestica* cv. 'Abbasi' affected by BA factor *in vitro*.

Table 3. Combined effects of IBA and NAA on rooting of *Malus domestica* cv. Abbasi micro-shoots *in vitro*.

| NAA (mg l ⁻¹) | IBA | Rooting (%) |
|------------------------------|-----|----------------|
| 1 | 0 | 73.86 a* |
| 0 | 1 | 49.88 abc |
| 0.5 | 0 | 66.00 a |
| 0 | 0.5 | 29.70 bc |
| 0.5 | 0.5 | 40.38 abc |
| 0.3 | 1.2 | 67.40 a |
| 0 | 2.4 | 61.16 ab |
| 0 | 3.3 | 19.44 c |

*The data with the same letters are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).

rooting of apple cv. 'Abbasi' *in vitro* shoots. Table 3 showed that NAA was more effective than IBA in rooting micro-shoots. Application of 1 (Figure 7, b) and 0.5 mg L⁻¹ NAA induced 73.86 and 66% rooting, respectively, and IBA induced 49.88 and 29.70% rooting, respectively. The lowest The optimization results of the factor values, based on the important responses of the shoot regeneration stage in apple cv. Abbasi, showed that the optimum combination concentrations for BA, IBA and GA₃ are 1.5, 0.1 and 0.02 mg L⁻¹, respectively (Figure 6).

and thick shoot production of the Greensleeves cultivar. They stated that BA at 8.8 and 17.6 μM induced swollen and deformed shoots. High concentrations of BA decreased the length of the internodes and increased the amount of vitrification that may have contributed to the shoot deformation (Karimpour *et al.* 2013).

Optimization

Experiment 2: Rooting

ANOVA showed the significant effect ($p \leq 0.05$) of auxins combination on the

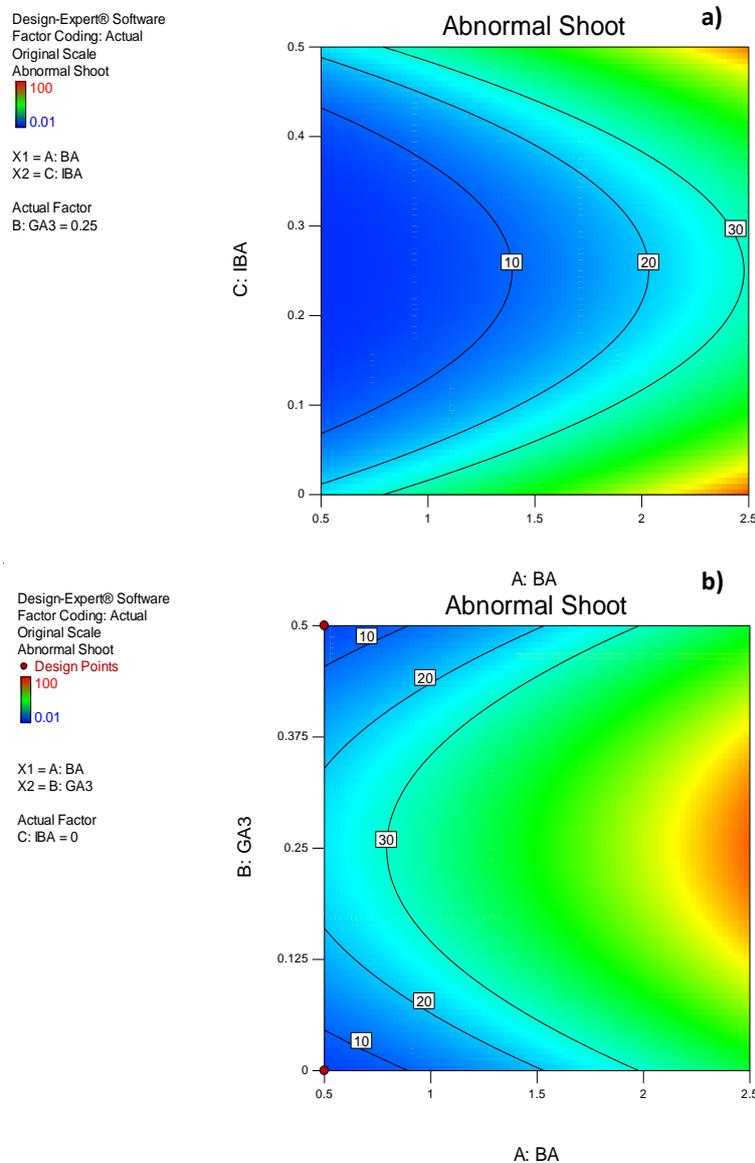


Figure 5. The abnormal shoot characteristics of *Malus domestica* cv. 'Abbasi' as affected by BA, IBA (a) and GA_3 *in vitro*.

rooting of apple cv. 'Abbasi' *in vitro* shoots. Table 3 showed that NAA was more effective than IBA in rooting micro-shoots. Application of 1 (Figure 7, b) and 0.5 mg L^{-1} NAA induced 73.86 and 66% rooting, respectively, and IBA induced 49.88 and 29.70% rooting, respectively. The lowest concentration of IBA (0.5 mg L^{-1}) could not retrieve the negative effect of the lowest

concentration of NAA (0.5 mg L^{-1}) on rooting (40.38%) but the highest concentration of IBA (1.2 mg L^{-1}) in combination with the lowest concentration of NAA (0.3 mg L^{-1}) induced 67.40% rooting. Similar to our results, with increasing IBA concentration the rooting percentage decreased in the M.26 rootstock but MM.106 rootstock was poorly rooted and

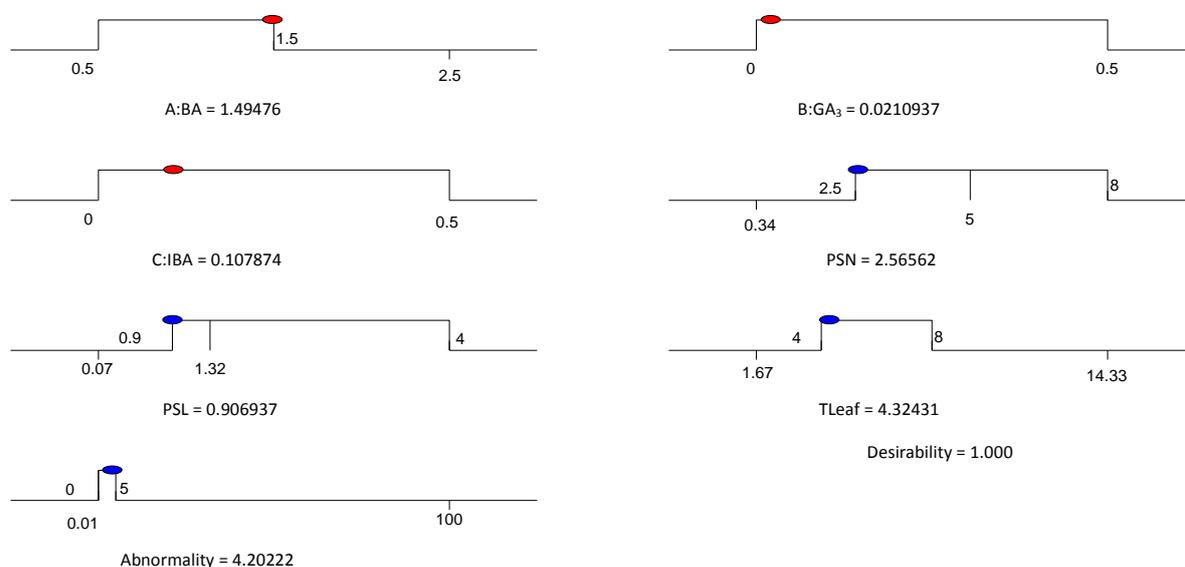


Figure 6. Optimal values were predicted by Design Expert for three factors of BA, IBA and GA_3 for proliferated shoot number, proliferated shoot length, leaf number and abnormal shoots of *Malus domestica* cv. 'Abbasi' shoots in *in vitro*.

unaffected by the IBA treatment (Magyar-Tabori *et al.* 2002b). Amiri and Elahinia (2011) also reported the effectiveness of IBA (1 mg L^{-1}) and NAA (0.5 mg L^{-1}) combination for M.9, M.27 and 'MM.106' rooting. Similarly, Miri (2018) stated that NAA has a greater impact on the rooting of M.9 and M.26 rootstocks than IBA, and the combination of IBA+NAA can improve rooting percentage at lower concentrations. Using NAA and IBA for the apple *in vitro* rooting showed different results depending on the genotype. Apple rootstocks M.27, M.26 and MM.111 at 0.02 to 6.6 mg L^{-1} NAA rooted better than the Macspur cultivar, however, the rootstock M.9 produced no roots (Lane and McDougald 1982). The Akero cultivar had 100% rooting using 0.5 mg L^{-1} IBA (Welander 1985). In vitro application of IBA and NAA had no significant effect on Royal Red Delicious, Gala, Golden Delicious and Spur McIntosh, while IBA significantly induced the

rooting in the Delicious, Redspur Delicious, McIntosh and Mutsu cultivars and NAA induced better rooting than IAA in the three cultivars of Delicious, McIntosh and Spartan (Zimmerman and Fordham 1985). In the other study, IBA was more effective than NAA for the MM.106 rooting (Sharma *et al.* 2000). The concentration of 1.5 mg L^{-1} of IBA induced maximum rooting in Azayesh-Esfahan, Morabei Mashhad and M.9 rootstocks (Ghanbari 2014).

The different stages of apple micropropagation cv. Abbasi are illustrated in Figure 7. The rooted shoots were transferred to pots containing cocopeat : perlite (1:1) mixture after removal of agar from the root medium and rinsed with water. After six weeks of adaptation in the growth chamber (Figure 7, c), they were transferred to the larger pots and kept in the greenhouse (Figure 7, d).



Figure 7. *Malus domestica* cv. 'Abbasi' in different stages of *in vitro* propagation: a) shoot proliferation in PMI medium (1.6 mg l^{-1} BA + 0.27 mg l^{-1} IBA + 0.01 mg l^{-1} GA₃); b and c) rooted shoot induced by 1 mg l^{-1} NAA; d and e) 'Abbasi' plantlets after 6 weeks (in the laboratory) and three months (in the greenhouse) of adaptation, respectively.

Conclusion

Response surface methods evaluate the interactions between multiple factors for one or more characters and aim to optimize responses based on the studied factors. In this experiment, the Design Expert software was successfully used to optimize the amount of plant growth regulators including BA, IBA and GA₃ for the shoot proliferation-related responses of apple cv. 'Abbasi'. The number of regenerated shoots was linearly and positively affected by BA, whereas leaf number response was linearly and negatively affected. The effect of GA₃ on the shoot length was linear and negative. The percentage of abnormal shoots in a quadratic polynomial model was affected by all three factors, BA, IBA and GA₃, while the response to BA was positive and linear, and the response to IBA and GA₃ were positive and negative, respectively in a quadratic model. According to the results of this experiment, optimum concentrations for BA, IBA and GA₃ were 1.5 , 0.1 and 0.02 mg L^{-1} ,

respectively. The production of normal shoots with a high regeneration rate appears to be more influenced by the quantity (amount) and quality (ratio) of the mineral combination rather than PGRs (Reed *et al.* 2013a; Reed *et al.* 2013b; Wada *et al.* 2013a; Wada *et al.* 2013b; Wada *et al.* 2015; Karimpour *et al.* 2020). Therefore, to improve the quantity and quality of the shoots produced during the shoot proliferation phase, complementary experiments including optimization of salts and other components of the medium such as vitamins and environmental factors should also be designed and evaluated by the response surface methods. The highest rooting percentage of apple cv. 'Abbasi' shoots were obtained using 1 mg L^{-1} (73.86%) and then 0.5 mg L^{-1} NAA (66%).

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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کاربرد روش سطح پاسخ به منظور بهینه‌سازی غلظت تنظیم‌کننده‌های رشد برای پرآوری شاخساره سیب رقم 'عباسی' در شرایط درون‌شیشه‌ای

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چکیده

بهینه‌سازی پروتکل‌های ریزازدیادی با اهداف تجاری و اصلاحی انجام می‌گیرد. امروزه تکنولوژی‌های کامپیوتری روش‌های جدیدی برای درک بهتر نقش عوامل درون‌شیشه‌ای در رشد گیاهان فراهم آورده است. به‌منظور بهینه‌سازی ساختن تنظیم‌کننده‌های رشد (GA₃, IBA, BA) در پرآوری سیب رقم 'عباسی' آزمایشی با سه فاکتور با استفاده از نرم‌افزار Design Expert و در قالب ۲۰ نقطه طراحی و اجرا گردید. آزمایش ریشه‌دهی در قالب طرح کاملاً تصادفی با ۸ ترکیب اکسین (NAA + IBA) پیاده شد. تعداد شاخه باززایی شده (۳-۵/۵) شاخه به ازای ریزنمونه به صورت خطی و مثبت تحت تأثیر فاکتور BA (۵/۵-۲/۵ میلی‌گرم بر لیتر) قرار گرفت، درحالی‌که پاسخ تعداد برگ (۳۳-۶۷/۱ میلی‌گرم بر لیتر) شاخه تولید شده به صورت خطی و منفی متأثر از BA (۵/۵-۲/۵ میلی‌گرم بر لیتر) بود. اثر GA₃ (۵/۵-۰/۰ میلی‌گرم بر لیتر) بر پاسخ طول شاخه (۳۲-۰/۰۷ سانتیمتر) به صورت خطی و منفی دیده شد. در صد شاخه‌های غیر نرمال (۱۰۰-۰/۰۱ در صد) در قالب مدل چندجمله‌ای درجه دوم تحت تأثیر هر سه فاکتور BA (۵/۵-۲/۵ میلی‌گرم بر لیتر)، IBA (۵/۵-۰/۰ میلی‌گرم بر لیتر) و GA₃ (۵/۵-۰/۰ میلی‌گرم بر لیتر) قرار گرفت، به این‌صورت که به‌صورت خطی و مثبت تحت تأثیر BA قرار داشت و به‌صورت درجه دوم مثبت و منفی به ترتیب از IBA و GA₃ تأثیر گرفت. مطابق نتایج این آزمایش غلظت بهینه برای IBA, BA و GA₃ به ترتیب ۱/۵، ۰/۱ و ۰/۰۲ میلی‌گرم بر لیتر می‌باشد که باعث تولید شاخه‌هایی با نرخ پرآوری ۲/۵ شاخه به ازای ریز نمونه، طول شاخه ۰/۹ سانتیمتر، ۴/۳ عدد برگ به ازای هر شاخه تولید شده و ۴ درصد شاخه‌های غیر نرمال می‌شود. در صد نکروزه شدن نوک شاخه متأثر از فاکتورهای مورد ارزیابی نبود. در ریشه‌دهی ریز شاخه‌ها، NAA از IBA مؤثرتر بود و بالاترین در صد ریشه‌دهی با کاربرد ۱ و سپس ۵/۵ میلی‌گرم بر لیتر NAA (به ترتیب ۷۳/۸۶ و ۶۶ درصد) به دست آمد.

واژه‌های کلیدی: ریزازدیادی؛ نرم‌افزار Design Expert؛ IBA؛ GA₃؛ BA؛ *Malus domestica* Borkh.