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Original Article

The effect of growth regulators and explants on callus induction in four cultivars of potato (*Solanum tuberosum* L.)

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Potato
Solanum tuberosum L.

Abstract

The effect of growth regulators and explants on callus induction of leaf and internode explants in potato (*Solanum tuberosum*_L.) was studied. Explants of four potato varieties were incubated in the Murashige and Skoog (MS) medium supplemented with various concentrations of growth regulators. The results show that the effects of genotype, type of explants, and growth regulators on measured characters were highly significant. The percentage of callus induction ranged between 44.16 and 98.38%. The highest rate of callus induction and the biggest fresh weight recorded for 5 mg/L NAA, 1 mg/L BA, and 1 mg/L GA3 in Bartina and Spunta variety respectively in both explants intermodal and leaves, and the best callus growth was obtained in internodal explants.

Mots clés :
Callogènese
culture *in vitro*
pomme de terre
Solanum tuberosum L.

Résumé

L'effet des régulateurs de croissance et de type d'explants sur la callogènese chez quatre variétés de pommes de terre (*Solanum tuberosum* L.). L'effet des régulateurs de croissance et des explants sur la callogènese pour les feuilles et les entre nœud chez la pomme de terre (*Solanum tuberosum* L.) à été étudié. Des explants de quatre variétés de pomme de terre ont été cultivés dans le milieu de Murashige et Skoog additionné de diverses concentrations de régulateurs de croissance.

Les résultats montrent que l'effet du génotype, du type d'explants et des régulateurs de croissance sur les caractères mesurés étaient très significatifs. Le pourcentage du callogènese variait entre 44,16 et 98,38%, le taux d'induction et le poids frais les plus élevés sont enregistré pour 5 mg / L NAA, 1 mg / L BA et 1 mg / L GA3 pour la variété *Bartina et* pour la variété *Spunta* respectivement, chez les deux explants, entre nœud et les feuilles. La meilleure croissance des cals a été obtenue chez les explants d'entre nœud.

INTRODUCTION

Potato (*Tuberosum solanum* L.) is the fourth most important food crop in the world following rice, wheat, and maize (Young-Min *et al*., 2013), is one of the most economically important annual vegetable crop of Solanaceae family (Solmon-Blackburn and Baker, 2001).mainly due to its starch content and high quality protein substantial amounts of essential vitamins, minerals, and very low fat content (Abd El-Moneim *et al*., 2012). Originating in the Andean countries and it is consumed as staple food in more

than forty countries in the world, which is ranked second after cereals as well (Arakawa *et al*., 1999).

The term "somaclonal variation" is used with reference to the observed variation at regenerated plants from the tissue and culture cells. It is known that the somaclonal variations can take place at isolated protoplasts, calli (nistor *et al*., 2009).

In vitro regeneration depends on various factors, the alteration of which may improve culture efficiency. Not only the change of media and use of various culture conditions, but also the choice of the tissue source for the explant, provides an opportunity to gain the optimal regenerating configuration (Janowicz *et al*., 2012).

Callus induction is important to produce genetic variability, which is vital in breeding program, and in addition, it is easy for producing a huge amount of *in vitro* plantlet via callus (Haque *et al.,* 2009). Thus, the research was conducted to investigate the *in vitro* callus initiation ability of potato with optimum concentration of plant growth regulators from the different plant parts,

Production of calli form from fragments of the plant are mainly carried out to determine the culture conditions required by the explants to survive and grow, study cell development (Samoylov *et al*., 1998),

The present study was designed to develop a callus induction protocol of potato genotypes from leaves and internodes explants, and to identify the suitable explants, genotype and culture medium for large scale utilization in tissue culture technique.

MATERIALS AND METHODS

Desiree, Spunta, Bartina and Kondor genotypes were obtained from SAGRODEV-ALGERIA. The sprouts of potato varieties were rapidly washed in 70% alcohol and then immersed in 0.1% $HgCl_2$ solution for 15 min and then washed in sterilized water for several times and they are placed in MS medium. *In vitro* plant materials of potato developed using nodes were grown on medium consisting of Murashige and Skoog basal medium (Murashige and Skoog, 1962). Leaves and internodes of microplant (4 to 5 cm in height) were cut into small pieces (2 to 5 mm) and cultivated on MS medium supplemented with different growth regulators by different concentrations: 5 mg/L NAA and 0.5 mg/L kinitin for medium 1 (M1); 5 mg/L NAA, 1 mg/L benzyldimethyldodecylammonium bromide (BAB), and 1 mg / L GA_3 for medium 2 (M2); 3 mg / L 2,4D and 0.5 mg / L kinitin for medium 3 (M3). Culture was kept for 30 days in a growth room at 25±2°C having 1.83 m fluorescent tubes and was illuminated 16 h daily with a light intensity of 1500 lux.

Calli characteristics

The following callus characteristics were measured:

(i) Callus induction

(ii) Percentage of callus induction: It was evaluated for four weeks (suitable for sub-culturing) (number of explants producing callus)/ (number of explants plated in tubes).

(iii) Fresh weight of callus after four weeks.

Statistical analysis

Statistical analysis focused on comparing different treatments using analysis of variance (ANOVA) with a threshold of 5%, followed by a comparison of means (LSD) and that if the interaction between the three factors was significant. The statistical analysis was carried out by using the Winstat software. Then, the results were represented graphically according to varieties, explants and media, using software Excel 2007.

RESULTS

In order to evaluate effect of growth regulators and explants on callus morphogenic and growth, the results were recorded for leaves and internodes explants from four potato genotypes growing on a basal MS media supplemented with three different combinations of growth regulators. Data were analyzed after four weeks of culture and the analysis showed that the percentage of callus induction, callus color and fresh weight varied with culture medium, explants and genotype. Callus quality varied from 'intermediate' to 'friable' in type and produced roots, although the roots were not very long less than 1 cm. The calli from the four varieties were light yellow and dark, especially for the M2. Calli were also different in consistency, compact or crumbly texture, smooth or rough (Figure 1).

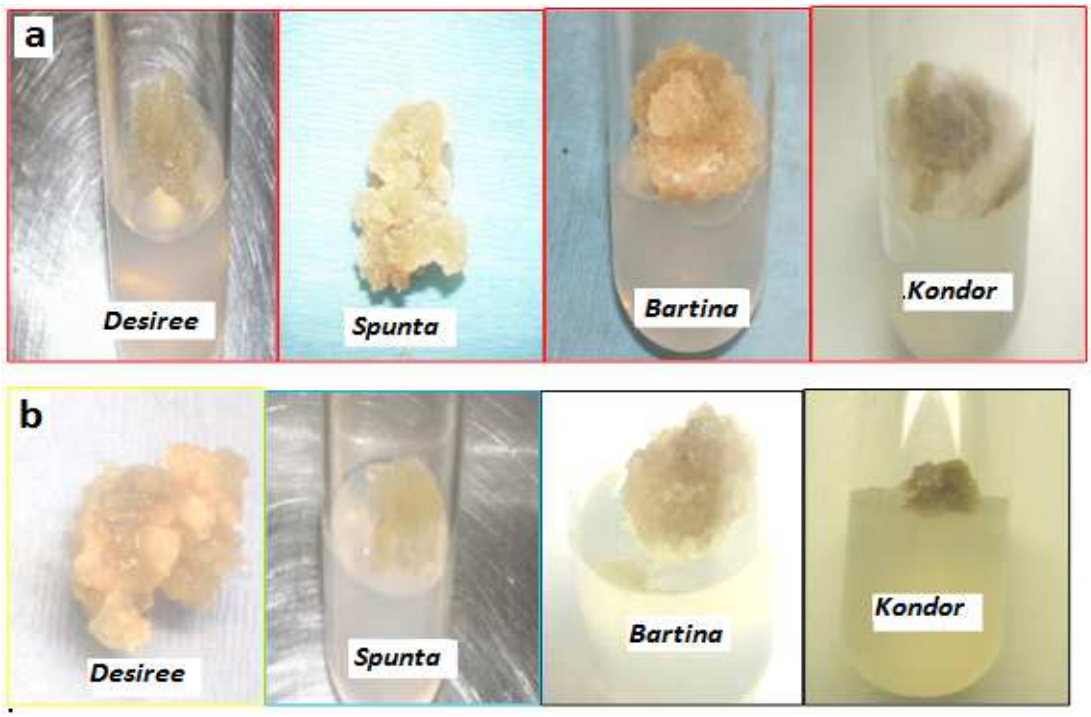

Figure 1. Morphogenic effect of genotype and explants on callus of potato in MS medium supplemented with 5 mg/L NAA, 1 mg/L BAB, and 1 mg/L GA3 after four weeks of culture. **a:** Callus Morphogenic induction from internodal explants of potato (*Solanum tuberosum* L.) and **b:** Callus morphogenic induction from leaves explants of potato cultivars.

Callus induction response

Callus induction response occurred on all media combinations (Figure 2). Final observations after 10 to 15 days showed that M1, M2 and M3 proved to be most efficient in callus induction response in maximum explants with maximum degree of callus induction. It observed the positive answer of both explants for callus induction .

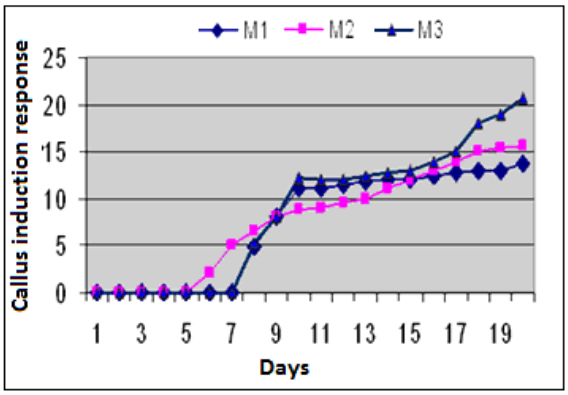

Figure 2. The effects of culture medium on callus induction response for all varieties. **M1**=medium1: 5 mg/L NAA and 0.5 mg/L kinitin, **M2**=medium2: 5 mg/L NAA, 1 mg/L BAB, and 1 mg / L GA3, **M3**=medium3: 3 mg / L 2,4D and 0.5 mg / L kinitin for medium 3 (M3).

From Figure 3, it may be observed that, callogenesis was developed after five days from internodal explants, and after seven days from leaves' explants for all varieties. In order to evaluate the effect of genotypes on callus induction response, the data was analyzed, analysis showed that the effect of genotypes on measured characters were found to be highly significant ($p<0.01$).

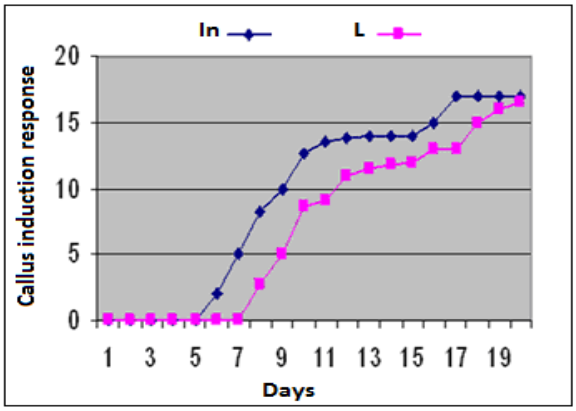

Figure 3. The effects of explants type on callus induction response for all varieties. **In:** Internode **and L:** leaves.

The highest callus induction response recorded was for Bartina variety incubated more than five days, which was followed by other varieties with seven days, with the continuation of callogenesis up to the 10th day for Bartina variety and to the 20th day for other varieties (Figure 4).

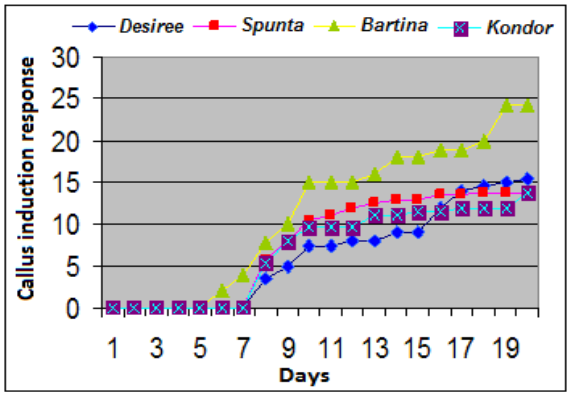

Figure 4. The effects of genotype on callus induction response.

Rate of callus induction

In order to evaluate the effect of culture medium and genotypes on the rate of callus induction, data analyzed showed that the effect of medium and genotypes on measured characters was found to be highly significant ($p<0.01$). For Desiree variety, the highest rate of callus induction recorded was 98.38% in M3, which was followed by 75.38% in M2 and 44.16% in M1. For Spunta variety, the highest rate of callus induction recorded was 81.66% in M3, followed by 43.28% in M2 and 36.66% in M1. However, for the other two varieties, Bartina and Kondor, similar rate of callus induction was recorded in all medium for Bartina, in M2 and M3 for Spunta (Figure 5). Internodal explants produced significantly the highest rate of callus induction for all the cultivars, while in contrast; the lowest rate of callus induction attributed to leaves explants for all varieties. Except in the Spunta variety, this showed 40% for leaves explants and 33.33% for internodal explants in M1 and M2. While for Desiree and Kondor varieties in M3, highest rate was observed for the leaves explants in compare with internodal explants (Figure 5).

M3 present a high effect (82%) on the rate of callus induction (Figure 6); by comparison with M2 witch present 70% and M1 with 55% only. The Figure 7 presents the effect of genotype on the rate of callus induction; Bartina was with a higher rate (95%), in comparison with: Desiree genotype (72%), *Kondor* genotype (57%), and *Spunta* genotype (55%).

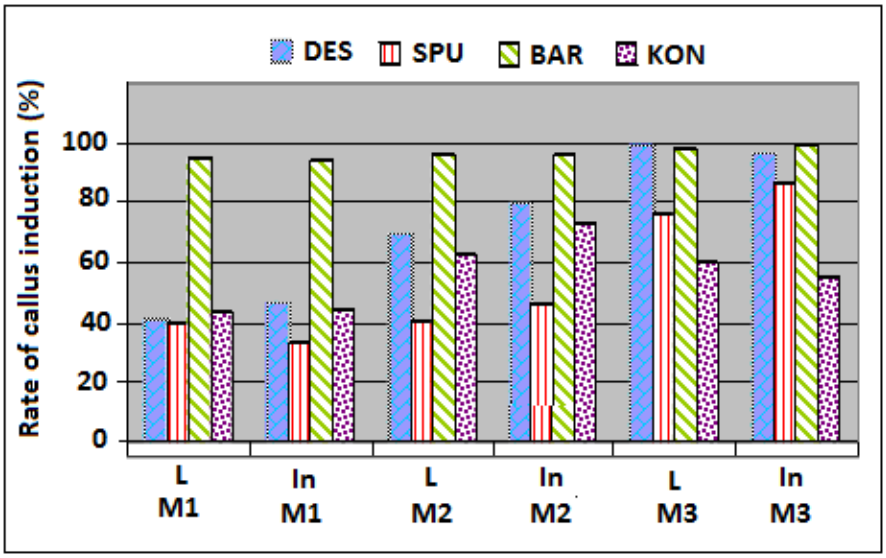

Figure 5. The effects of culture medium, genotype and explants on the rate of Callus Induction. **DES**= Desiree, **SPU**=Spunta, **BAR**=Bartina and **KON**=Kondor; **L**=leaf explants, **In**=internodal explants; **M1**=medium1: 5 mg/L NAA and 0.5 mg/L kinitin, **M2**=medium2: 5 mg/L NAA, 1 mg/L BAB, and 1 mg / L GA3, **M3**=medium3: 3 mg / L 2,4D and 0.5 mg / L kinitin for medium 3 (M3).

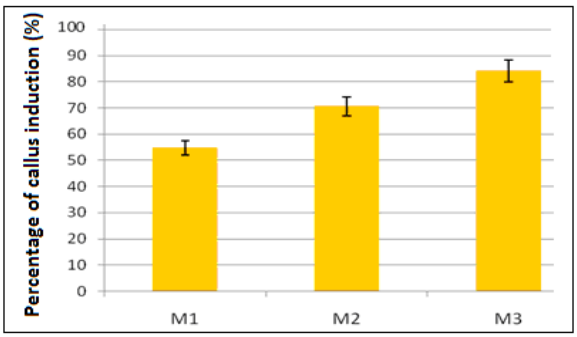

Figure 6. The effects of culture medium on the rate of callus induction.
M1=medium1: 5 mg/L NAA and 0.5 mg/L kinitin, **M2**=medium2: 5 mg/L NAA, 1 mg/L BAB, and 1 mg / L GA3, **M3**=medium3: 3 mg / L 2,4D and 0.5 mg / L kinitin for medium 3 (M3).

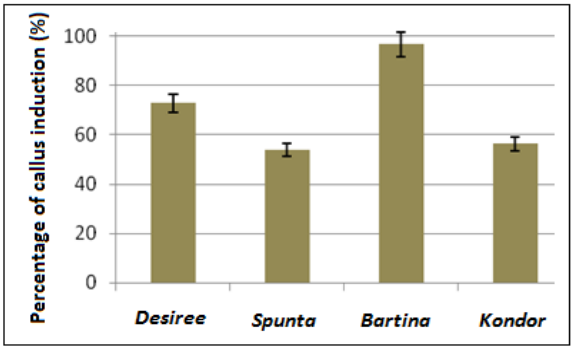

Figure 7. The genotype effects on percentage of callus induction.

Figure 8 presents the effect of explants on the rate of callus Induction, intermodal explant present a higher rate (71%), in comparison to leaves explants (68.5%).

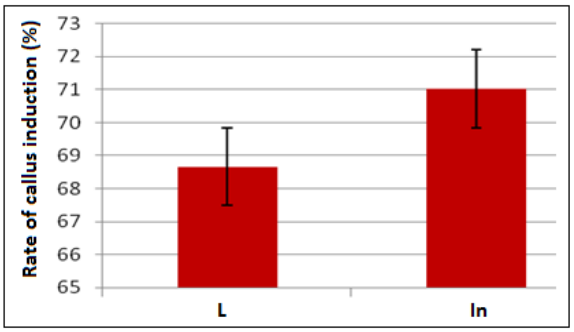

Figure 8. The explants effects on the rate of callus induction.
In: Internode, **L:** leaves

Fresh weight

The callus fresh weight has provided a more concise quantitative character for the development rate of callus. In order to evaluate the effect of culture medium, explants, and genotypes on callus growth, the data were analyzed and the analysis showed that the effect of medium and genotypes on character measured were found to be highly significant ($p<0.01$). We can observe (Figure 9) the effect of growing regulators on fresh weight, the biggest fresh weight was observed on M2 for *Spunta* variety in both explants intermodal and leaves, for Bartina and Kondor varieties in intermodals explants, this was M3. The results were more important for the Desiree variety for both explants (Figure 9). The best callus growth was obtained in internodal explants while in contrast, the lowest callus growth was attributed to leaves explants for all varieties. With the influence of genotype on callus growth, we could record that the highest callus growth was from Bartina and Desiree's varieties.

DISCUSSION

Callus formation was considered an important means for the application of plant biotechnology (Nguyen *et al*., 2002). A number of research projects have indicated the relationship between the callus induction and culture conditions in different potato species (Charlotte *et al*., 1987; Yasmin *et al*., 2003; Hakan 2004; Khadiga *et al*., 2009) cotton (Anita and Bhojwani 1976), the soybean (André *et al*., 2003), sweet potato (Chen, 1987), rice (Abeyaratne *et al*., 2004; Kabir *et al.*, 2008). Callus initiation on cut ends of *in vitro* cultured potato explants could be observed in leaves, after 10 days in MS supplemented with concentrations similar to those we used (Charlotte *et al*., 1987). However, other findings were reported by Khadija *et al*. (2009), that callus initiation on cut ends of *in vitro* cultured potato explants (Diamond variety) could be observed after seven days in culture medium similar to our medium. In this study, the highest response of callus induction in all the varieties for both leaf and internodals' explants were observed in M3, M2 and M1. This observation concurs with previous studies of Yasmin *et al*. (2003) which has shown that 2.5 mg / L NAA and 0.5 mg / L BA were effective in callus induction and among all concentrations, only 2.5 mg / L NAA with 2 mg / L BA and 1.25 mg / L NAA with 1 mg / L BA was found to be the most effective concentration for callus induction of 95 and 90%, respectively, in all potato cultivars used.

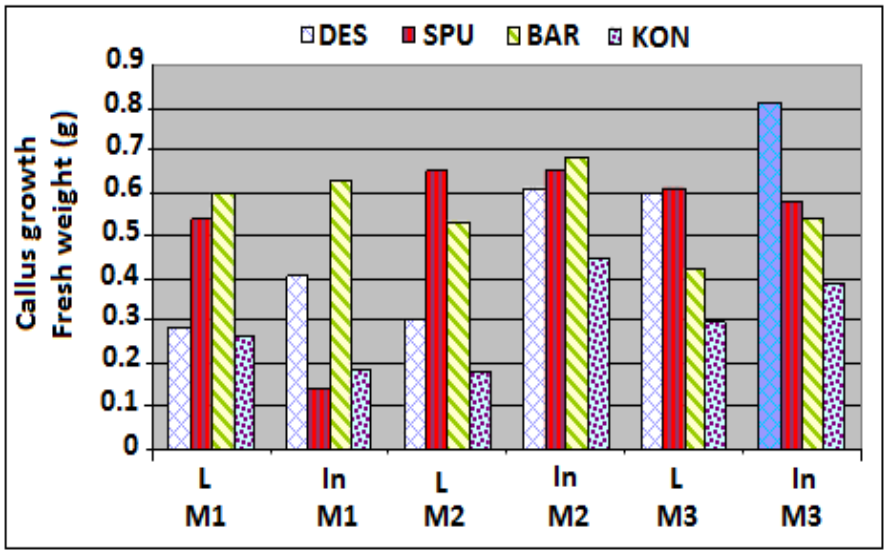

Figure 9. The effects of culture medium, genotype, and explants on Callus growth: Fresh weight DES=Desiree, SPU=Spunta, BAR=Bartina and KON=Kondor; L=leaf explants, In = internodal explants, M1=medium1: 5 mg/L NAA and 0.5 mg/L kinitin, M2=medium2: 5 mg/L NAA, 1 mg/L BAB, and 1 mg / L GA3, M3=medium3: 3 mg / L 2.4D and 0.5 mg / L kinitin for medium 3 (M3);

Hakan (2004) has found that the MS media supplemented with the highest concentration of NAA with BA (M2) produced the best potato callus. Khadiga *et al*. (2009) has performed best result in the MS medium supplemented with 3 mg / L 2,4-D and 2 mg / L BA (M3) was effective in inducing potato callus in Diamond variety. This study proves that the culture media used in our experiments have a different effect on potato callogenese. This is in agreement with Houri *et al*. (1990) where the composition and concentration of growth regulators in the culture media affect the amount of callus formation, and characteristics of the developing cells. The result on genotype explants and culture conditions effect on callusing ability is in agreement with all previous studies (André *et al*., 2003; Khadiga *et al*., 2009). The growth regulators were necessary for callus induction from internodes and leaf explants of four potato cultivars. However, other findings were reported by Yasmin *et al*. (2003); Khadiga *et al*. (2009), then the explants grown in culture medium without growth regulators did not produce callus. The combination of a cytokinin with an auxin has been reported to strongly enhance callus induction in potato.

Furthermore, this study reveals that leaves and internodals explants responded differently to callus induction at different rates with internodes having the highest percentage of callus induction in all the media. This showed that internodals explants have a better response to callus induction than leaf explants in all the varieties used in the study and it is in line with earlier reports that regeneration of sweet potato occurs at different frequencies for various explants (Liu and Cantliffe, 1984; Dhir *et al*., 1998). Therefore, this study demonstrates that different genotypes require different protocols for callus induction, though not forgetting the effect of growth regulators (Charlotte *et al*., 1987). Bartina formed calli at all concentration for both leaf and internodals explants, unlike the other varieties. This demonstrated that different varieties respond differently to callus formation. This as well confirms earlier reports, that callus induction is influenced by the genotype. In addition, this study proves that callus induction ability was greatly influenced by the genotype used.

CONCLUSION

The focus of this study was to establish an efficient method for callus induction of potato. It was observed that the combination of NAA, BA, and GA3 produced excellent results in terms of callus induction.

1. The medium which induced callus in the highest percent was M2, containing NAA (5 $mg.L^{-1}$), BA (1 $mg.L^{-1}$), and GA3 (1 $mg.L^{-1}$).
2. The genotype which had the highest influence over callus induction was Bartina.
3. The explants which had the highest influence over callus induction were internodals explants.

ABBREVIATIONS

BA, 6, benzyl-adenine; **NAA,** α-naphthalene acetic acid; **IBA,** indole-3 butyric acid; **IAA,** indole-3 acetic acid; **GA_3,** gibberellic acid; **2,4-D,** 2,4-dichlorophenoxyacetic acid.

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