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Assessing the response of indigenous loquat cultivar Mardan to phytohormones for in vitro shoot proliferation and rooting*

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Abstract: In vitro cultures of loquat cultivar Mardan were established using shoot apices after treating with NaOCl (5%, 7%, 10%, 12%, 14% (v/v)) for 12 min and $HgCl_2$ (0.01%, 0.05%, 0.10%, 0.20%, 0.25% (w/v)) for 2 min. A maximum survival rate of 70% was recorded after surface sterilization with 10% NaOCl. Caulogenic response was assessed on Murashige and Skoog (MS) medium fortified with assorted combinations of the cytokinins, benzylaminopurine (BAP), kinetin, and N6-(2-isopentyl)adenine (2iP). Treatment of BAP 1.5 mg/L combined with 2iP 9.0 mg/L and kinetin 1.5 mg/L was found to be optimum for shoot morphogenesis in terms of the number and subsequent growth of shoots, while the highest shoot length was yielded by the combination of BAP 0.5 mg/L, kinetin 0.5 mg/L, and 2iP 3 mg/L. Higher levels of cytokinins induced callogenesis, vitrification and stunted growth to some extent. For rhizogenesis, uniform sized micro-shoots were excised and transferred to half-strength MS medium containing auxins. The best rooting expression was observed with naphthaleneacetic acid (NAA) 1 mg/L combined with indole-3-butyric acid (IBA) 2 mg/L and paclobutrazol (PBZ) 1 mg/L.

Key words: *Eriobotrya japonica*, Micropropagation, Sterilization of loquat, Plant growth regulator, Shoot proliferation, Rhizogenesis

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1 Introduction

Loquat (*Eriobotrya japonica* Lindl.) is an important evergreen fruit crop of subtropical regions. It is grown in more than thirty countries (Feng *et al*., 2007) with China having the leading share (Lin, 2007). It is a popular fruit in Pakistan and is available to satisfy consumer needs in spring when no other fruit is available in the market, thus fetching good prices. However, until recently (Hussain *et al*., 2007), it has been neglected in terms of research. In Pakistan, most of the loquat orchards were established using seeds due to the unavailability of certified and true to type planting stock in local nurseries. Consequently, there is a lack of uniformity among plants giving low quality fruits with low yield. Cultivation of superior genotypes of loquat may increase production, thereby increasing availability for the home market and for export (Hussain *et al*., 2007). Loquat cultivar Mardan has large, round, and attractive fruit with an orange yellow skin and sweet tasting pulp (Hussain, 2009) which can help it to capture local and export markets.

Plant cell, tissue, and organ culture techniques are becoming an integral part of propagation systems for year-round supply of clonal plant material on a mass scale (Al-Sulaiman and Barakat, 2010) in a very short time span, which is impossible by conventional approaches (Barakat, 2008). Their application will also satisfy sanitary and phytosanitory (SPS) demands in the context of the World Trade Organization (WTO). The current lack of certified germplasm repositories and seasonal fluctuations in supply

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require immediate application of in vitro techniques to overcome these problems of the fruit industry. Biotechnological tools for mass propagation of different tree species have been introduced recently, particularly in the last decade (Azad *et al*., 2005; George *et al*., 2008), and micropropagation protocols are available for many species and cultivars (Ruzic and Vujovic, 2008). However, propagation of woody plants in vitro is an intricate process. Clonal propagation of loquat using in vitro techniques has been carried out earlier (Lomtatidze *et al*., 2009), but there is a need to optimize cultivar specific protocols as no trials have been made for indigenous loquat genotypes. Shoot tip culture is highly reliable and is the preferred technique for obtaining disease-free stock (Panjaitan *et al*., 2007), while production of vigorous shoots with a rapid rate of multiplication is one of the prerequisites of reliable micropropagation practice. In vitro root development for subsequent acclimatization is another important factor determining the success of micropropagation. Cytokinins are a class of plant growth regulators (PGRs) involved in many processes of plant growth, including cell division and shoot and root morphogenesis. In particular, PGRs are known to regulate axillary bud growth. Auxins have a cardinal role in coordination of many growth processes in a plant's life cycle and are essential for plant root development (Nordstrom *et al*., 2004). Considering the importance of these PGRs, this study aimed to evaluate different types and concentrations of cytokinins and auxins for in vitro shoot development and rhizogenesis in loquat.

Protocols standardized following this study will be conducive for the micropropagation of elite loquat genotypes on a commercial scale, which will finally uplift the national horticulture industry and export potential.

2 Materials and methods

Shoot tips (3–4 cm in size) of loquat cultivar Mardan were collected from the orchard of Tret (Murree, Pakistan) to be used as explants for in vitro culture establishment. After removing leaves, hairs, and dirt, they were placed under running tap water with one drop of Tween 80 and detergent for 30 min to remove any foreign contaminants. After washing, shoot apices were dissected and surface sterilized with different concentrations of NaOCl (5%, 7%, 10%, 12%, 14% (v/v)) for 12 min and $HgCl_2$ (0.01%, 0.05%, 0.10%, 0.20%, 0.25% (w/v)) for 2 min in a laminar air flow hood. Inoculation was done in culture jars containing Murashige and Skoog (MS) shoot proliferation media (MS macro, micro salts, and vitamins), supplemented with various combinations of cytokinins (BAP, 2iP, and kinetin) (Table 1). Data were recorded after 28 d for the number of shoots, shoot length (cm), number of leaves, and fresh and dry weights (mg).

Table 1 Different combinations of cytokinins for shoot proliferation of loquat cultivar Mardan

Treatment	Cytokinin (mg/L)		
	BAP	2iP	Kinetin
T_{C0}	0.0	0.0	0.0
T_{C1}	0.5	3.0	0.5
T_{C2}	1.0	6.0	1.0
T_{C3}	1.5	9.0	1.5
T_{C4}	2.0	12.0	2.0

BAP: benzylaminopurine; 2iP: N6-(2-isopentyl)adenine

For root induction, uniform sized (1.5 cm) micro-shoots were transferred to rooting medium (half strength of MS) supplemented with different levels of indole-3-butyric acid (IBA) in combination with naphthaleneacetic acid (NAA) and paclobutrazol (PBZ) (Table 2). Data were recorded after six weeks for rooting percentage, number of roots, and root length (cm).

Table 2 Different combinations of auxins for rhizogenesis in loquat cultivar Mardan

Treatment	Auxin (mg/L)		
	NAA	IBA	PBZ
T_{A0}	0.00	0.00	0.00
T_{A1}	0.25	0.50	0.25
T_{A2}	0.50	1.00	0.50
T_{A3}	0.75	1.50	0.75
T_{A4}	1.00	2.00	1.00
T_{A5}	1.25	2.50	1.25

NAA: naphthaleneacetic acid; IBA: indole-3-butyric acid; PBZ: paclobutrazol

The experiment was designed as a single factorial with three replications for each treatment and five plants per replication. Data were statistically

analyzed using the analysis of variance (ANOVA) (MSTAT-C software) and means were compared using the least significant difference (LSD) test at 5% α level (Steel *et al*., 1997).

3 Results and discussion

3.1 Culture establishment

Surface sterilization of loquat is a very difficult step in culture initiation due to its hairy nature. Furthermore, exudation of phenolics and browning are other common problems associated with its in vitro culture, as with other members of the Rosaceae family (Bhojwani and Razdan, 1983; Amin and Jaiswal, 1987). NaOCl is the most suitable disinfectant for a variety of plant species. It plays a significant role in eliminating microorganisms from the surface of explants (Canli and Kazaz, 2009). However, the efficacy of surface sterilization can be improved by standardizing the most appropriate combination of dose and exposure time of disinfectants (Yildiz and Er, 2002).

3.2 Effects of NaOCl and $HgCl_2$ on in vitro culture establishment of loquat cultivar Mardan

Data regarding the effect of disinfectants on surface sterilization of loquat shoot tips after 28 d are presented in Fig. 1. Treatment with 10% NaOCl showed significant results with an increased survival percentage (70%). Treatment with 14% NaOCl resulted in minimum survival (2%) and maximum necrosis (90%) (Fig. 1a). Generally, necrosis increased as the concentration of NaOCl increased which shows that higher concentrations of NaOCl damage young tissues. Fungal and bacterial contamination was high (86.66%) after treatment with 5% NaOCl, but absent after treatment with 14% NaOCl. The highest NaOCl concentration badly damaged the explants and decreased the survival rate.

$HgCl_2$ significantly reduced contamination (to 3.33%) after treatment of 0.25% $HgCl_2$ but increased mortality (to 53.3%). Percentage survival was highest (63.33%) after treatment of 0.10% $HgCl_2$ followed by 0.20% $HgCl_2$ (43.33%) (Fig. 1b). The percentage survival after treatments of 0 and 0.05% $HgCl_2$ was not significantly different (40%). Increases in the concentration of $HgCl_2$ showed increasing necrosis and mortality, but a decline in contamination. $HgCl_2$ is one of the strongest disinfectants used in culture establishment. The action of $HgCl_2$ may be through lysis of cells of microorganisms or it may act on thiol groups in microbial enzymes. Like NaOCl, $HgCl_2$ is known to be a powerful antimicrobial agent (Russell and Chopra, 1990). Treatment with 0.10% $HgCl_2$ for 5–7 min is used commonly for surface sterilization of many plant species (Skirvin *et al*., 1986). El-Zaher (2008) obtained good results with 0.30% $HgCl_2$ for 5 min in jackfruit. Our results are in line with the findings of Chavan *et al*. (1996) who stated that $HgCl_2$ at 0.20% for 4 min or at 0.10% for 10 min gave good sterilization results in shoot tip cultures of jackfruit.

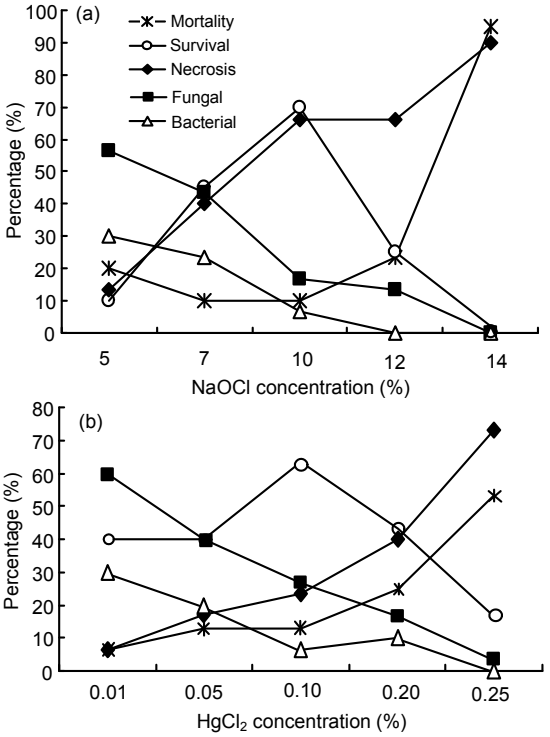

Fig. 1 Effects of different levels of NaOCl (a) and $HgCl_2$ (b) on culture establishment of loquat cultivar Mardan

Comparison of the two sterilizing agents showed that NaOCl gave a maximum survival rate of 70% and a minimum mortality rate of 10% after treatment of 10% NaOCl, while $HgCl_2$ gave a maximum survival rate of 63.33% after treatment of 0.10% $HgCl_2$ and a minimum mortality rate of 6.66% after treatment of 0.01% $HgCl_2$. From the above results, we infer that

treatment with NaOCl at 10% performed better than treatment with $HgCl_2$. NaOCl is also a readily available and economical sterilant.

3.3 Effects of cytokinins on shoot development

3.3.1 Number of shoots

Different combinations of cytokinins (BAP+2iP +kinetin) interacted significantly in terms of the number of shoots (Fig. 2). Treatment T_{C3} (BAP 1.5 +2iP 9.0+kinetin 1.5 mg/L) yielded the maximum number of shoots (4.84) followed by T_{C4} (BAP 2.0 +2iP 12.0+kinetin 2.0 mg/L) at 3.25 (Fig. 3a). Increasing BAP doses in combination with 2iP+kinetin had an increasing effect up to a certain level (T_{C3}), after which a declining trend was observed in shoot growth. Our findings are in accordance with the results of Al-Maarri and Al-Ghamdi (1995), who reported that application of BAP and 2iP produced a good response for initiation and multiplication of shoot tip cultures in date palm. Histological studies showed that the inclusion of BAP in shoot proliferation media enhanced the growth of axial shoots and promoted the multiplication of shoots from the basal tissues of explants (Ohki and Sawaki, 1999). The successful use of BAP for shoot production may be accredited to the capability of plant tissues to metabolize BAP more efficiently than other synthetic PGRs or to the capacity of BAP to encourage the production of natural hormones, such as zeatin, within the tissues (Malik *et al*., 2005). Kukreja *et al*. (1990) and Vincent *et al*. (1992) also reported a decline in the number of shoots with higher BAP levels. Waseem *et al*. (2009) showed that the use of higher than optimal concentrations of PGRs may lead to poor performance.

Rapid growth and multiplication of shoots are based on the quantity and quality of cytokinins and auxins in media as well as on their endogenous levels in plants, which vary from species to species and between growth phases (Panjaitan *et al*., 2007). BAP manipulates shoot production by stimulating rapid cell division to induce multiple shoot formation (Roy and Banerjee, 2003; Ronzhina, 2003). Lane (1992) reported that shoot production was improved in response to BAP application. In olive, 2iP at 10 mg/L produced a high number of shoots (Agrawal *et al*., 1991). Similarly, Al-Sulaiman and Barakat (2010) documented that the presence of 2iP in the medium at 10.0 mg/L had a positive effect on the production of lateral shoots and other growth parameters of *Zizi-phus spinachristi*. Increases in shoot number might be related to the application of an appropriate dose of cytokinin which promotes bud growth and overcomes apical dominance (Asaad *et al*., 2009).

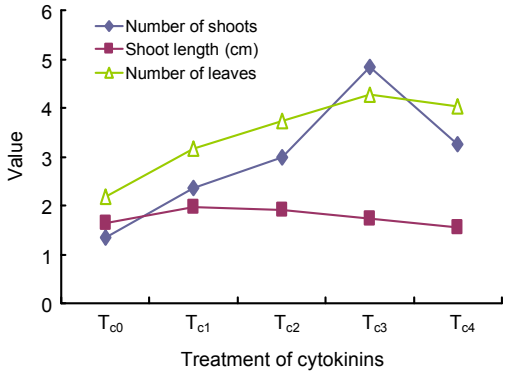

Fig. 2 Effects of different combinations of cytokinins (BAP, 2iP, and kinetin) on in vitro shoot proliferation of loquat cultivar Mardan

Fig. 3 T_{C3} (BAP 1.5+2iP 9.0+kinetin 1.5 mg/L) exhibiting maximum number of shoots (i.e., 4) (a) and maximum shoot length (3 cm) at T_{C2} (BAP 1.0+2iP 6.0 +kinetin 1.0 mg/L) (b)

3.3.2 Shoot length

Data presented in Fig. 2 show that the shoot length was markedly affected by various combinations of cytokinins. Statistically, longer shoots (1.97 and 1.92 cm) were obtained with treatments T_{C2} (BAP 1.0+2iP 6.0+kinetin 1.0 mg/L) and T_{C1} (BAP 0.5+2iP 3.0+kinetin 0.5 mg/L), respectively, followed

by T_{C3} (BAP 1.5+2iP 9.0+kinetin 1.5 mg/L) (1.74 cm) (Fig. 3b). After treatment T_{C4} (BAP 2.0+2iP 12.0 +kinetin 2.0 mg/L) with elevated levels of cytokinins, shoot length decreased to 1.56 cm, demonstrating the negative impact of increased doses of PGRs as reported by Carelli and Echeverrigary (2002). Cytokinins release apical dominance, which hastens axillary bud formation and reduces the length of explants (Huetteman and Preece, 1993). Increased BAP levels in shoot multiplication media suppress shoot growth due to the regeneration of multiple shoots (Waseem *et al*., 2009). Singh and Arora (1995) similarly reported that increasing BAP concentrations increased the number of shoots but depressed the shoot length in chrysanthemum. At minimum levels, cytokinins produce longer shoots, which may be due to their reduction in number. Baig *et al*. (2011) also found that BAP is a strong cytokinin which reduces shoot length and increases the number of side shoots. Exposure of cultures to higher concentrations of BAP during regeneration of shoots may lead to an increase in cytokinins, which inhibits further shoot growth (Malik *et al*., 2005). In woody plants, BAP is paramount for growth compared to kinetin (Fráguas *et al*., 2004). The frequency of shoot multiplication and the length and number of shoots depends mainly on the optimal level of BAP, after which response declines (Waseem *et al*., 2009). Higher than optimal concentrations of BAP enhance ethylene production in the base of plants which may block the transport of exogenous auxin in the basal portion of explants and result in minimum shoot growth and development (Ahmad *et al*., 2003). Yakimova *et al*. (2000) stated that cultured plants producing large numbers of shoot buds exhibit minimum shoot length because all the nutrients are utilized for the formation of lateral shoots.

The best shoot elongation was observed with 0.6 mg BAP in olive (Ahmad *et al*., 2003). BAP is preferable to other cytokinins for promoting shoot production and proliferation (Siddique and Anis, 2009). BAP is degraded very slowly in culture medium which makes it available to plant tissues for a longer period of time (Hussain *et al*., 2011).

3.3.3 Number of leaves

The maximum number of leaves (4.26) was observed after treatment T_{C3} (BAP 1.5+2iP 9.0+kinetin 1.5 mg/L) followed by T_{C4} (BAP 2.0+2iP 12.0 +kinetin 2.0 mg/L) (Fig. 2). We infer from the results that the combination of cytokinins in treatment T_{C3} is optimum for better performance, as the shoot number, and therefore the number of leaves, increased with this treatment. In contrast, the leaf count was meager (2.16) of the control treatment, confirming the significance of these phytohormones for leaf growth.

2iP and BAP are important PGRs for bud break and shoot differentiation due to their role in cell multiplication and the breakdown of apical dominance (Casimiro *et al*., 2001). Multiplication of shoots depends mainly on the number of buds and leaves on an explant. Specific plant hormones may arrest or hasten the growth and development of leaves. Previously, it was indicated that BAP had a strong effect on promoting the growth of leaves and bud initials, causing a rise in the number of bud primordia in chrysanthemum (Karim *et al*., 2002; 2003). Kapchina-Toteva and Yakimova (1997) reported that peroxidase activities increased following cytokinin application, leading to the sprouting of axillary buds which subsequently developed into leaves. Various scientists have studied the role of cytokinins in bud formation and leaf growth (Mok, 1994).

3.3.4 Fresh and dry weights

Results showed that the maximum fresh weight (200.55 mg) was obtained after treatment T_{C3} (BAP 1.5+2iP 9.0+kinetin 1.5 mg/L) followed by treatments T_{C4} (198.88 mg, BAP 2.0+2iP 12.0+kinetin 2.0 mg/L), and T_{C2} (190.12 mg, BAP 1.0+2iP 6.0 +kinetin 1.0 mg/L). The control treatment produced the minimum fresh weight (171.51 mg) (Fig. 4). The combination of BAP, 2iP, and kinetin at 1.5, 9.0, and 1.5 mg/L, respectively, proved to be optimum for the fresh weight of shoots, which is consistent with the results for other parameters (i.e., the number of shoots per explant and the number of leaves). Fresh weight is correlated with the quality of shoots. After treatment T_{C3}, the shoots were quite healthy and had an excellent leaf:stem ratio but after treatment T_{C4} some vitrified plants were observed (Fig. 5), which reduced the total fresh weight of the plants. Moreover, the decrease in fresh weight after treatment T_{C4} was due to reduced shoot and leaf number at this level. Vitrified plants are deficient in chlorophyll content and show hyperhydricity, hypolignification, and changes

in enzyme activity and protein synthesis, which can cause alterations in normal plant metabolic processes (Ziv, 1992). These results support those of Li *et al*. (2003) who proposed that the combination of high concentrations of cytokinins with a high water potential in the medium is one of the major causes of vitrification.

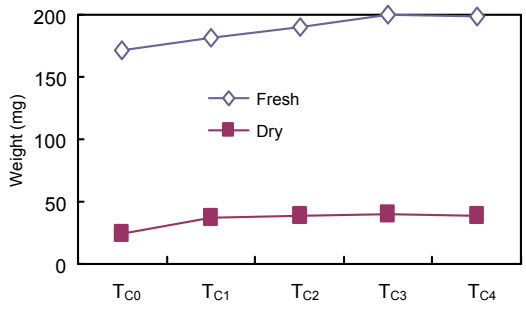

Fig. 4 Effects of different combinations of cytokinins (BAP, 2iP, and kinetin) on fresh and dry weights of loquat cultivar Mardan

Fig. 5 Vitrified plants with stunted growth at higher levels of cytokinins (T_{C4}, BAP 2.0+2iP 12.0+kinetin 2.0 mg/L)

Dry weight showed synergism with fresh weight. The maximum accumulation of dry weight (40.04 mg) was after treatment T_{C3} (BAP 1.5+2iP 9.0+kinetin 1.5 mg/L) followed by T_{C4} (BAP 2.0+2iP 12.0 +kinetin 2.0 mg/L) (39.13 mg). A declining trend was observed in dry matter as the number of shoots and leaves decreased, as for fresh weight. The highest dry weight was found after treatments T_{C3} and T_{C4}, which we attribute to there being the same amounts of shoots, leaves, and biomass accumulation. Barberaki and Kintzios (2002) reported that the addition of macronutrients in plant tissues is significantly affected by the addition of PGRs. Kinetin and BAP enhance leaf area and maximum dry weight accumulation (de Oliveira *et al*., 2010). Increases in cytokinin levels lead to increases in the numbers of leaves and shoots, and in the rooting percentage by triggering cell division and elongation (Schmulling *et al*., 2003). Baig *et al*. (2011) studied the role of BAP in dry matter accumulation and reported that it may enhance physiological vigor, leading to the conversion of more sugars into dry matter in different regions of explants.

Significant increase in dry weight may be related to sucrose concentrations in media. Sucrose is incorporated into structural carbohydrates in cell walls, which form the major part of a plant's dry weight (Gollagunta *et al*., 2004). Moreover, dry weight is affected by an increase in enzyme activities coupled with the production of more shoots. An enhancement of peroxidase activity in response to cytokinins and in association with an increased number of open axillary buds was reported in cultured *Rosa hybrida* in vitro (Yakimova *et al*., 2000). The activity of enzymes is induced by high cytokinin concentrations, due to an elevation of the RNA levels from a subset of the genes involved (Taiz and Zeiger, 2002). According to Vuylsteker *et al*. (1997), the fresh weight of a plant depends on the number of shoots and therefore it may decline due to a reduced shoot number. Bennet *et al*. (1994) reported that a decrease in fresh weight might arise because kinetin is less effective than BAP in prompting the enzyme activity involved in promoting vegetative growth. High concentrations of cytokinins cause senescence of tissues, possibly due to the production of ethylene, which consequently gives lower fresh weight (George *et al*., 2008).

3.4 Effects of auxins on root development

3.4.1 Number of roots

Different combinations of auxins significantly affected the number of roots. The maximum number (12.06) was obtained after treatment T_{A4} (NAA 1.00 +IBA 2.00+PBZ 1.00 mg/L) (Fig. 6b) followed by T_{A5} (NAA 1.25+IBA 2.50+PBZ 1.25 mg/L) (10.34) (Fig. 7). An increasing trend was observed with increasing concentrations of auxins up to the level of treatment T_{A4} but after that there was a slight reduction in root number with T_{A5}. Rooting response was highly impoverished in the control treatment (T_{A0}).

Fig. 6 Average root length (10 cm) (a) and maximum number of roots (12.06) (b) at T_{A4} (NAA 1.00 +IBA 2.00+PBZ 1.00 mg/L)

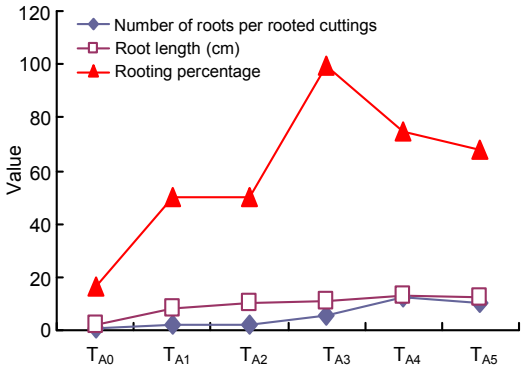

Fig. 7 Effects of different combinations of auxins (NAA, IBA, and PBZ) on in vitro rooting of loquat cultivar Mardan

Auxins play a vital role in regulating the growth and multiplication of plant organs (Woodward and Bartel, 2005; Teale *et al*., 2006) and in lateral root formation (Ruegger *et al*., 1997; Rogg *et al*., 2001). IBA is the most common auxin, and is the strongest, most highly stable and least toxic rooting plant hormone among all auxins (Weisman *et al*., 1988; Hartmann *et al*., 2007). George *et al*. (2008) also found that development and growth of roots is accelerated by auxins, which enhance cell division in the root zone. Root initiation is dependent on the interaction between external and internal factors, such as the type and level of endogenous PGRs. However, auxin is one of the important factors (Asaad *et al*., 2009). The application of IBA causes changes in the synthesis of protein and the production of RNA. These changes lead to more rapid cell growth and division and, as a result, the number of roots increases (Husen and Pal, 2007). PBZ belongs to the triazole family which includes hormones that act as plant multiprotectants against stresses (Gilley and Fletcher, 1997). The role of PBZ in root initiation is still unknown, but it acts as an antigibberellin in plants (Kefford, 1973). Various scientists have reported that application of PBZ increases antigibberellin compounds, which may promote root production (Kefford, 1973; Tim *et al*., 1985).

In the present study, we observed that high levels of auxins induced callogenesis in the basal part of shoots and exerted an inhibiting effect on root growth and development. IBA at higher levels enhances degradative metabolism in tissues and inhibits root initiation and development (Baker and Wetzstein, 1994). The findings of Baker and Wetzstein (1994) also suggest that auxins at higher than optimal concentrations may lead to reductions in the number of roots and in the rooting percentage.

3.4.2 Root length

Root length of the loquat genotypes was influenced by the various types and combinations of auxins (Fig. 7). Maximum root length (13.07 cm) was obtained after treatment T_{A4} (NAA 1.00+IBA 2.00 +PBZ 1.00 mg/L) (Fig. 6a) followed by T_{A5} (NAA 1.25+IBA 2.50+PBZ 1.25 mg/L) (12.33 cm).

With increasing concentrations, auxins greatly promoted root length but at the highest concentrations of NAA, IBA, and PBZ, root length decreased. Auxin concentration is most important for root elongation which may be inhibited by increasing levels of exogenous auxins in the rooting media (Baker and Wetzstein, 1994). Cell multiplication and proliferation are responsible for fresh tissues in the root multiplication zone that direct the regeneration of roots around the periphery of the quiescent region, consequently enhancing root length (Hopkins, 1995). Hu and Wang (1983) reported that the root elongation stage is very responsive to auxin concentration and may be inhibited by higher concentrations.

PBZ application, for in vitro rooting of plants, increases resistance against desiccation (Roberts and Matthews, 1995). Auxins intensify adventitious root initiation due to production of endogenous enzymes, which may hasten cell division, differentiation, and multiplication (Husen and Pal, 2007).

High concentrations of auxin (IBA) reduce root length and number below optimal levels. Ozel and Arslan (2006) reported that they also raise the endogenous auxin levels in plants providing protection against catabolism and increasing interactions with growth inhibitors by conjugation. Plants with long roots and higher root numbers are produced, which is probably due to effect of endogenous hormones (George *et al*., 2008). Taiz and Zeiger (2002) also found that lower concentrations of auxin promote root growth but higher levels reduce growth due to the production of ethylene in the root zone, which acts as a growth inhibitor. Baker and Wetzstein (1994) observed that the production of degradative metabolites increases with increasing concentrations of IBA, which blocks the root regeneration progress (Rai *et al*., 2009).

3.4.3 Rooting percentage

Significant differences (P<0.05) were observed among different auxin treatments in terms of rooting percentage (Fig. 7). The highest rooting percentage (99.71%) was recorded after treatment T_{A3} (NAA 0.75+IBA 1.50+PBZ 0.75 mg/L) followed by treatments T_{A4} (NAA 1.00+IBA 2.00+PBZ 1.00 mg/L) (74.930%) and T_{A5} (NAA 1.25+IBA 2.50+PBZ 1.25 mg/L) (67.667%). However, the minimum rooting percentage (16.66%) was recorded in the control treatment. Data showed that IBA at 1.50 mg/L with NAA and PBZ each at 0.75 mg/L was the optimum combination. The rooting percentage showed a decreasing trend with higher concentrations of auxins. Auxins are effective PGRs which enhance the process of root growth and multiplication through the differentiation of vascular tissues (Asghar *et al*., 2011). Among auxins, IBA is known to stimulate rooting more efficiently due to its weak toxicity and greater stability for root induction (Han *et al*., 2009). The rooting percentage is increased by increasing the auxin levels, but at higher concentrations the number of roots is decreased (Asghar *et al*., 2011). Auxins encourage rooting through biochemical changes in the plant system (Henrique *et al*., 2006). Bhatt and Tomar (2010) found that IBA at a minimum concentration produced the best results.

Differentiation of root primordia due to an optimum concentration of IBA, enhances cambial development at the base of explants, which increases the maximum rooting percentage (Haq *et al*., 2009). Efficient changes in IBA genes can promote auxin biosynthesis and then alter root development, while PBZ can reduce stem growth due to an antigibberellin effect (Rademacher *et al*., 1984; Graebe, 1987). PBZ inhibits gibberellin synthesis and ethylene production, and increases cytokinin production in roots (Kamountsis and Chronopoulou-Sereli, 1999). Auxin stimulates the production of growth substances for root development and elongation (Han *et al*., 2009).

4 Conclusions

The current study documents the protocol optimization for Loquat micropropgation. Results reveal that sodium hypochlorite is an effective sterilant for surface disinfection of loquat shoot tips in vitro. Moreover, this article gives standardized doses of PGRs to achieve considerably higher shoot and root growth for mass scale, healthy and clonal production of this economically important fruit crop. It will consequently lead to the establishment of certified loquat nurseries in the scenario of SPS measures of WTO.

Compliance with ethics guidelines

Nadeem Akhtar ABBASI, Tariq PERVAIZ, Ishfaq Ahmed HAFIZ, Mehwish YASEEN, and Azhar HUSSAIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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