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Effect of mineral concentration on *in vitro* explant growth of almond (*Prunus amygdalus* var. Binazir)

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Abstract

A study was undertaken to determine the potential of mineral dependent growth of almond *in vitro.* Shoot-tip of almond (*Prunus amygdalus* L. var. Binazir) was subcultured on four different concentrations (4, 6, 8, 10 gl^{-1}) of gelled modified de Fossard medium (de Fossard, 1976) with four relative concentrations (0X, 0.2X, 1X and 2X basal medium) containing BA 0.75 mgl^{-1} and NAA 0.75 mgl^{-1}. As mineral concentration increased, both growth and multiplication rate significantly (P=0.05) increased. But increase was not proportional. There was a negative relationship between mineral concentration and root formation. Agar concentration affected the percentage of root formation and hyperhydration. The greatest amount of growth (fresh weight 29%, and dry weight 0.30%) were obtained in the high (2X) mineral concentration with low agar (6 gl^{-1}) treatment after 8 weeks culture period. The highest multiplication rate (7-8 number $month^{-1}$) was also obtained in the same treatment (2X mineral and 6 gl^{-1} agar concentrations). No hyperhydration was observed in high agar concentration treatments. This means, increasing agar concentration resulted in decreased hyperhydration phenomenon, however, growth and multiplication rate decreased as agar concentration increased. Highest percentage (68%) of root formation was obtained in low mineral and low agar concentration treatment. Multiplication rate was 2-4 $month^{-1}$ at low (0.5X) concentration and increased to 7-8 at high (2X) concentration.

Key words: Hyperhydration, medium composition, multiplication, root formation, tissue culture, *Prunus amygdalus*

Introduction

Almond (*Prunus amygdalus* L.) is one of the main nut fruits. It is traditionally propagated by seedling, budding or grafting on to seedling which is laborious and slow. Non uniform germination, prolonged seedling emergence, and disease susceptibility to mycoplasma are other problems related to tradtional propagation (Hammerschlag, 1986; Hartmann *et al.*, 1990). Many woody species are able to regenerate a whole plant from an *in vitro* cultured shoot-tip (Murashige and Huang, 1987). "Shoot tip culture" techniques have been used for the accelerated growth and multiplication rate and forms an important and advantageous tool for rapid mass propagation of disease-free plants. But there are still certain problems which limit its widespread use for almond (Rugini *et al.,* 1987). One of the major shortcomings of shoot tip culture technique in almond is low quantity and quality of growth and occurrence of hyperhydration (translucency) (Vieitez *et al.,* 1985; Rugini *et al.,* 1987; Pasqualetto *et al.,* 1988). Hyperhydration (succulency and glassiness) refers to a physiological and morphological disorder in tissue culture grown plants. It is a major problem in the tissue culture industry since it can affect shoot multiplication and culture vigour (Hammerschlag, 1986) and can impede the successful transfer of micropropagated plants to *in vivo* conditions. Up to 60% of affected plants fail to acclimatise (Paques and Boxus, 1987).

Hyperhydricity could be controlled by agar concentration (Debergh *et al.,* 1981; Paques and Boxus, 1987; Ghashghaie *et al.,* 1991). There is strong connection between the culture medium hardiness, the proliferation rate and vitrification. Lowering the nutrient gel hardiness increased the proliferation and vitrification rate of artichoke (Debergh *et al.,* 1981). Hyperhydration can be caused by high concentrations of minerals (nitrate ammonium) (Pasqualetto *et al.,* 1988). The gelling agent used in the medium can be another factor inducing hyperhydration *e.g.* Gelrite induces hyperhydration in apple (Pasqualetto *et al.,* 1988). Rugini *et al.* (1987) reduced vitrification drastically in almond replacing sucrose by fructose (45 gl^{-1}); Paques and Boxus (1987) avoided hyperhydration in liquid or solid medium with BAP (1 mgl^{-1}). They used a hydrosoluble agar fraction called "antivitrification complex". These materials are complex polysaccharides which, when dissolved in hot water or ionic solution, form cross-links between the macromolecules to create a solid medium (Williams, 1993). Physical parameters of gelled media such as water potential, medium conductivity and solute diffusion, which are responsible for better growth, are affected by gel brand and its concentration (Williams, 1993). Inherent properties (the chemical and physical) which control the availability of water and hence growth quality (hyperhydration), is related to gel concentration (Smith and Spomer, 1994; Williams, 1993).

Although the gelling agents reduce the incidence of hyperhydricity they have inhibited explant growth, compared to growth on liquid medium (Amiri, 2000). In this work, the effects of medium composition (minerals and gelling agent) were examined on almond.

Materials and methods

To measure the effect of medium constituents (minerals and gelled agent) on growth quantity (fresh and dry weight) and growth quality (plant appearance and hyperhydration) of almond, four relative mineral concentrations (0X, 0.2X, 1X and 2X basal medium) were used with de Fossard medium (de Fossard, 1976),

along with four different concentrations (4, 6, 8, 10 gl^{-1}) of agar (Bacto BiTeck agar). These treatments were supplemented with BA 0.75 mgl^{-1}. and NAA 0.75 mgl^{-1}, sucrose 3%, thiamine, myo-inositol and L-tyrosine. pH was adjusted to 5.6 by HCl 0.5 N and NaOH 0.5 N before autoclaving. Then medium was autoclaved by 101 KPs, 120° C for 15 min. Thirty ml of solution in each 250 ml polycarbonate container was dispensed (with 5 replicates of each treatment and the control).

Four uniform shoot tips (3-5 mm) of almond (*Prunus amygdalus* L. var. Binazir) were cultured on each medium treatment and subcultured aseptically in different containers. All explants were kept in a growth room at a temperature of 25 ± 2°C, with 55% relative humidity and cool white fluorescent tubes with a light intensity ranging from 16-50 µmol m^{-2} s^{-1}.

Fresh and dry weights (as growth rate) and multiplication rate, percentage of rooting and hyperhydration were measured at 6th and 8th week. Containers did not occupy fixed positions on culture shelves but were moved around randomly during visual examination every week.

Data was analysed by ANOVA and means subjected to LSD test (P=0.05) NEVA (Burr, 1980). All calculations were performed by Microsoft Excel. Four shoot tips were inoculated per container and four containers were maintained for each treatment. Each unit in the container was considered a replicate and data were analysed for a factorial experiment involving four levels of agar and four levels of mineral treatments.

Results and discussion

Both growth (fresh and dry weights) and multiplication rate of almond explants were dependent upon medium composition (minerals and agar). As mineral concentration increased, both growth and multiplication rate significantly (P=0.05) increased. But increase was not proportional. Whereas, there was a negative relationship between mineral concentration and root formation (Table 1). Furthermore, gelling agent affected the percentage of root formation and hyperhydration (Table 1). The greatest amount of growth (fresh weight 29%, and dry weight 0.30%) were obtained in the high (2X) mineral concentration with low agar (6 gl^{-1}) treatment after 8 weeks culture period (Fig. 1). The highest multiplication rate (7-8 no mon^{-1}) was also obtained in the same treatment (2X mineral and 6 gl^{-1} agar concentrations) (Fig. 2). The highest percentage (43%) of hyperhydration was observed in high (2X) mineral concentration with very low agar (4 gl^{-1}) treatment. In other words, growth quality (hyperhydration) of almond explants was significantly influenced by both mineral and agar concentrations in the medium. For example, hyperhydration of explants was observed in high concentration (2X) of minerals in low gelled medium. The greater amount of multiplication rate, fresh weight and dry weight as final growth of almond explants in low agar-gelled medium (6 gl^{-1}) treatment compared to high agar-gelled (10 gl^{-1}) medium (Table 1) has been reported previously by many authors (Debergh *et al.*, 1981; Bornman and Vogelmann, 1984; Kordan, 1988; and Ghashghaie *et al.,* 1991). The greatest multiplication rate and highest amount of growth (fresh and dry weight) in high mineral concentration (2X) with low agar concentration (6 gl^{-1}) treatment correspond with higher total uptake of minerals and water availability. Low agar-gelled medium behaves differently from high gelled medium, in many manners like water potential, mineral solubility, mineral mobility and availability to the explant. In other words, plant growth *in vitro* depends on mineral uptake and mineral availability. Mineral availability to the explant depends on mineral solubility and

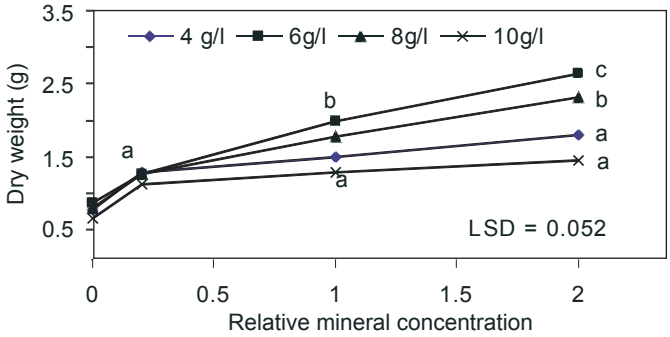

Fig.1. Final growth (dry weight) of four shoot-tips cultured almond (*Prunus amygdalus* L. var. Binazir) explants as affected by four levels (X0, X0.2, X1, and X2) of mineral supply and four levels (4, 6, 8, 10 agar gl^{-1}) of agar concentrations (at week 8).

Table 1. Effects of
and hyperhydration of almond (*Prunus amygdalus* L. var. Binazir) during 8 weeks culture.

Relative mineral concentration	Agar conentration (gl^{-1})	Fresh weight (g)	Dry weight (g)	Multiplication rate (number $month^{-1}$)	Rooting (%)	Hyperhydration (%)
0X	4	7.7	0.08	2.0	58	3.1
	6	8.6	0.09	2.5	56	0.0
	8	7.3	0.08	2.6	51	0.0
	10	6.0	0.07	2.0	46	0.0
0.2X	4	13.4	0.14	2.5	52	8.3
	6	13.1	0.14	3.5	47	6.2
	8	19.9	0.14	3.2	42	5.0
	10	26.9	0.12	2.4	38	0.0
1X	4	15.4	0.16	3.2	45	23.0
	6	21.2	0.22	6.0	40	13.4
	8	17.9	0.20	4.9	38	9.2
	10	12.6	0.14	2.8	30	5.3
2X	4	19.2	0.20	4.0	27	43.2
	6	28.8	0.30	7.8	23	28.8
	8	23.4	0.26	6.0	11	17.3
	10	14.4	0.16	3.0	2	12.8
LSD (P=0.05)		1.25	0.02	1.51	6.38	4.86

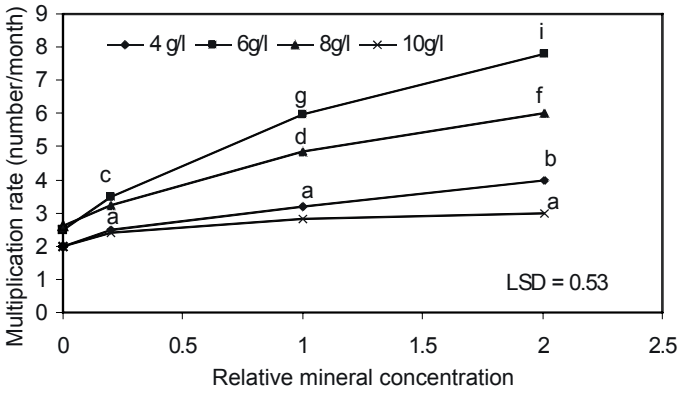

Fig. 2. Effect of four levels (X0, X0.2, X1, and X2) of mineral supply and four levels (4, 6, 8,10 agar gl^{-1}) of agar concentrations on multiplication rate of almond (*Prunus amygdalus* L. var. Binazir).

mineral transport through gel; both these depend on availability of free water (Amiri, 2000).

High rate of multiplication and non physiological disorders (hyperhydration) are the most important economical factors for successful mass propagation of almond. Although, increasing mineral concentration increased rate of multiplication, hyperhydration can be caused by high concentration of minerals (especially nitrate ammonium) (Pasqualetto *et al.*, 1988). Hyperhydration is reported to occur more often on rich (2X concentration) culture media such as Murashige and Skoog's (1962). The evidence of role of ammonium concentration on hyperhydration has been demonstrated by Letouze and Daguin (1983) on *Salix babylonica*. They induced hyperhydration in *Salix* by introducing an ammonium concentration equal to that of an ammonium MS medium into a Knop medium deprived of growth substances. These observations have been confirmed by the findings of Vieitez *et al.* (1985) on *Castanea sativa.* Furthermore, potassium and calcium ions concentration has been described much more higher in vitreous tissues than in normal ones (Pasqualetto *et al.,* 1988).

It can be concluded that improvements in micropropagation of almond is achievable by altering medium composition and water potential (agar and sucrose). It is possible to avoid hyperhydration and to reverse it if the phenomenon is not too advanced. Medium composition, especially, mineral concentrations and gelling agent, must be carefully adjusted.

Acknowledgments

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