

Full Length Research Paper

Influence of medium composition on multiplication of walnut (*Juglans regia* L.) growth

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***In vitro* proliferation of walnut (*Juglans regia* var. *zeiabadi*) was studied in response to the four levels (1/3X, 1/2X, 1.0X and 1.5X) of macrominerals of DKW (Driver and kunjyukj, 1984) medium. Macrominerals (N, P, K and Mg) can be a limiting factor for growth quantity (fresh weight and dry weight) and growth quality (explants appearance) of walnut explants. Addition of 0.5X macrominerals to the basal DKW medium in which containing 4.0 µM 6-benzylaminopurine (BA) and 12 µM indole-3-butyric acid (IBA), significantly (P = 0.05) improved the proliferation. Better response of was found with 1.5X macrominerals concentration in respect of growth and proliferation. In contrary, the decrease in mineral availability observed in the depressed growth in the low macrominerals supply treatments (1/3X and 1/2X), (the restricted mineral availability was the main cause of the inhibition of the growth). However, root formation was worsted by supplemented additional minerals. For example, low mineral concentration treatment exhibit high rooting ability (75%) and high mineral concentration showed low (13%).**

Key words: Micropropagation, medium composition, walnut, mineral concentration.

INTRODUCTION

The most popular way of walnut (*Juglans regia* L.) asexual propagation is budding, which is labor intensive, time-consuming and costly. On the other hand, propagation by cuttings is very difficult due to their low rooting ability (McGranahan and Leslie, 1990). In the last decade, *in vitro* cultured techniques have been investigated for the successful large scale propagation of walnut. For example, Rodriguez et al. (1989) reported the establishment of walnut cultures *in vitro* and to describe the development of shoots or roots from cultured walnut embryos. Later, a large amount of works was conducted on different walnut species using different types of explants, media, and culture conditions and rooting techniques, with encouraging results (Jay-Allemand and Cornu, 1986; Gruselle et al., 1987; Cornu and Jay-Allemand, 1989). Most of the aforementioned work was based on a medium developed by DKW medium (Driver

and Kuniyuki, 1984) for the *in vitro* culture of *Juglans* species. Several reports indicate that *Juglans* species suitable to a certain degree, to micropropagation (Somers et al., 1982; Driver and Kuniyuki, 1984; Heile-Sudholt et al., 1986; Gruselle et al., 1987; Lee et al., 1986; McGranahan et al., 1988; Revilla et al., 1989; Felaliev, 1990; Leslie and McGranahan, 1992). Somatic embryogenesis has been induced from immature cotyledons in a number of species of *Juglans* (Cornu, 1988; Cornu and Jay-Allemand, 1989; Long et al., 1992; Neuman et al., 1993; Pijut, 1993a, 1993b) and shoot tips rooted plantlets which originated from cotyledon segments with embryonic roots and histology of root structures (Jay-Allemand et al., 1991; Tulecke and McGranahan, 1985; Polito et al., 1989) and also root formation of nodal segments and embryonic axes (Revilla et al., 1989); shoot formation of apical and lateral buds (Felaliev, 1990); bud formation and shoot multiplication (Gruselle et al., 1987; Stephens et al., 1990); somatic embryo originated from ovules improved acclimatization of plantlets (Voyiatzis and McGranahan, 1994).

Weekly transfer of butternut nodal explants to fresh

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Table 1. Effects of mineral concentration on growth (fresh and dry weight), multiplication rate and root formation of walnut (*Juglans regia* var. Zeiabadi) explants.

Rooting	Multi. Rate ²	DW	FW	Rel.min. conc. ¹
(%)	(no mon-1)	(g)	(g)	DKW medium
75	2.1	0.08	7.3	1/3X
52	2.9	0.14	12.9	1/2X
28	3.2	0.20	17.9	1.0X

¹mineral supplied of DKW medium (1/3X, 1/2X, 1.0X and 1.5X macroelements). ²Multiplication rat.no. mon⁻¹).

culture medium was necessary to maintain optimum growth and to limit the build up of phytotoxic exudates in the culture medium. The production of exudates from freshly cultured explants of walnuts has also been a problem, solved by employing explants presoaks and transferring explants frequently to fresh medium (Preece et al., 1989; Leslie and McGranahan, 1992). In contrast, DKW medium has proven to be suitable (and in many cases superior) for the culture of *J. regia* as well as other *Juglans* species (Driver and Kuniyuki, 1984; Heile-Sudholt et al., 1986; Lee et al., 1986; McGranahan et al., 1988; Leslie and McGranahan, 1992). As evidence show that many researches with *Juglans* spp. have focused on somatic embryogenesis and initial explant material for the purpose of clonal propagation and subsequent genetic improvement (Preece et al., 1989; Rodriguez et al., 1989). However, few reports have been published on the effects of mineral composition of *in vitro* cultured. Changes in the levels of mineral supply in the medium resulted to changes in the tissues of *in vitro* cultured plantlets are often associated with physiological disorder such as cessation (Barbas et al., 1993), or hyperhydric malformations (Kevers and gasper, 1986). In this work, it is shown that mineral is critical for the growth and root formation of walnut explants.

MATERIALS AND METHODS

One month after fruit set, the immature walnut (*Juglans regia* var. Zeiabadi) nut, was cracked and the kernel was immersed in NaOCl (1.0% w/v) for 5 min then rinsed three times in sterile distilled water. Embryo was carefully isolated from the kernel and it was established on gelled basal medium and was incubated in a growth chamber in darkness. After three weeks germinated embryos *in vitro*, four uniform size explants were established on four (1/3X, 1/2X, 1.0X and 1.5X) levels of macroelements of DKW (Driver and kunjyukj, 1984) medium, supplemented with indolbutric acid (IBA) 12 µM, benzyladenine (BA) 2 µM, sucrose 3%, agar (Difco BiTek™ agar) 8%. The medium pH was adjusted to 5.6 by HCl 0.5 N and NaOH 0.5 N before autoclaving.

All explants were kept in a growth room at a temperature of 25 ± 2°C, with 55% relative humidity and 16 h 50 µ m⁻¹ S⁻¹ cool white fluorescent light. Dry and fresh weights (g), multiplication rate (no. month⁻¹), root formation (%) and adventitious roots per shoot were recorded one month after transfer to new development medium. Containers did not occupy fixed positions on culture shelves but

were moved around randomly during visual examination every week.

RESULTS AND DISCUSSION

Result of this experiment revealed that different aspects of 'Zeiabadi' walnut growth were mineral dependence in culture medium. As macromineral concentration increased, both growth (dry and fresh weights) and multiplication rate significantly (P=0.05) increased. Whereas, there was a negative relationship between macromineral increase and root formation. The greatest amount of biomass (dry weight 0.26 g, fresh weight 23.4 g) and highest multiplication rate (4.6 no. month⁻¹) were obtained in the high (1.5X) macromineral supply treatment. In contrast, the highest percentage (78%) of root formation was obtained in low mineral concentration treatment (Table 1). In Table 1, the effects of different level of macromineral on all growth aspects (fresh and dry weights, multiplication rate, root formation) have been shown. As the mineral supply increased root formation decreased. Biomass production (shoots elongation) was dependent upon mineral supply in the medium. Regarding the number of the newly formed axillary shoots, the highest amount of growth was obtained in 1.5X DKW medium.

Although, higher concentrations of IBA (that is, containing 12 mM IBA) gave the best respond to root formation. As for the elongation, it seems that the best combinations of mineral and growth regulators were those of 12 µM IBA. Great variation (P=0.5) on rooting percentage, and total root length was observed among treatments (Figure 1).

It should be mentioned that treatment with low rooting ability developed fewer but longer roots compared with the high rooting ability clones which formed many main and secondary roots.

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Figure 1. Influence of mineral concentration on multiplication rate (No. explants/month) of walnut (*Juglans regia* var. Zeiabadi) cultured on gelled medium.

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