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Effect of explants density and size on the *in vitro* proliferation and growth of separated shoots of globe artichoke (*Cynara cardunculus* var. *scolymus* L.)

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- ✓ Multiplication rate

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ABSTRACT

We investigate here the effect of the density and the size of explants on the in vitro proliferation of globe artichoke shoots (*Cynara cardunculus* var. *scolymus* L.; accession 'Art 21'). For this purpose, we examined the rate of proliferation of separated shoots according to their sizes (< 1 cm, 1 to 1.5 cm, 1.5 to 2 cm and > 2 cm), and their densities (3, 4, 6 and 7 shoots per 132 cm2 area of culture media) in the proliferation medium containing 1mg.l-1 kinetin and 1 mg/l-1 NAA. The results showed that explants with a size comprised between 1-1.5 cm exhibited 100% rate of shoots survival and they give the highest number of new formed buds (7.33). In addition, we found that the density of 4 explants per 132 cm2 of culture medium area was the most suitable to promote budding and shoots proliferation and consequently increase the rate of multiplication.

1. Introduction

Globe artichoke (*Cynara cardunculus* var. *scolymus* L.) is an ancient herbaceous perennial plant, originating to the Mediterranean area, which is nowadays widely cultivated all over the world. Globe artichoke is an allogamous, diploid plant with 2n=2x=34 chromosomes [1] that produces well developed capitula, known for their high nutritional and medicinal value. Globe artichoke is propagated following two methods (or modes): a sexual method (using seeds) or an asexual method (by vegetative propagation). However, each of these methods has its limitation: the propagation by seeds results in the production of heterogeneous brood; while the vegetative multiplication depends on the mother plant healthiness. Although being the most used method in a big scale production [2], the vegetative propagation is still costly and results in a low multiplication rate (around 5 shoots by plant per year) [3-4].

We believe that the introduction of micropropagation could be an alternative to improving the health, the quality and the uniformity of clones. This will contribute positively to increasing the farming area and reducing the cost of growing the globe artichoke. Attempts for the micropropagation of the artichoke have begun in the early seventies. However, these attempts were confronted by experimental limitation due to the difficulty to sterilize the material used as source of explants, and also by the relative low rate of multiplication [5]. The use of *in vitro* propagation of globe artichoke, as a way of improving its rate of multiplication, was reported in several studies in recent years. However, these reports focused mostly on parameters related to the medium composition [6-7]; and the effect of growth regulators [3-8-9]; or on the genotypes [10-11-12] and the type of explants [13-14-15-9].

However, the effect of the explants density and size on the formation of artichoke shoots, under conventional micropropagation system, has not been reported to date. Our work intends to examine the outcome of the variation of explants density and shoot size on the shoot proliferation of the artichoke, and to evaluate the expected consequences that may have on its rate of multiplication.

2. Experimental details

2.1. Origin of explants and their sterilization

Seeds of *Cynara cardunculus* var. *scolymus* L. accession 'Art 21' were obtained from a local nursery (International Nursery, Tin Mansour, Agadir). The seeds were disinfected using 0.5% (w/v) $HgCl_2$ for 5 min, followed by 15 min treatment with 1% (v/v) NaOCl containing a few drops of Tween-20. After three washes with sterile distilled water under laminar air flux cabinet, the teguments were removed and the seeds were placed in glass tubes containing 20 ml of Murashige and Skoog (MS) medium [16], supplemented with 30 $g.l^{-1}$ sucrose and 0.3% phytagel for germination. The pH of the medium was adjusted to 5.6 prior to the addition of phytagel, then autoclaved at 121°C for 20 min. Cultures were incubated at 25±1°C with a photoperiod of 16h and a light intensity of 40 $\mu E.m^{-2}.s^{-1}$.

2.2. Culture establishment

MS medium (micro and macro elements), containing Gamborg's (B5) vitamins [17], 20 g/l sucrose as carbon source and growth regulators [1 $mg.l^{-1}$ indole-3-butyric acid (IBA), 0.1 $mg.l^{-1}$ gibberellic acid (GA_3)], was solidified with 0,3% phytagel for initial culture establishment of seedling [18]. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or with 0.1N HCl before autoclaving at 121 °C for 20 min.

2.3. Shoot induction and proliferation

After six weeks of culture, the explants were obtained by removing apical buds, leaves and roots from plantlets. These explants were transferred to fresh MS medium, supplemented with B5 vitamins, 20 $g.l^{-1}$ sucrose, 40 $mg.l^{-1}$ adenine sulfate, 50 $mg.l^{-1}$ of monosodium phosphate, 1 $mg.l^{-1}$ kinetin, 0.1 $mg.l^{-1}$ NAA and 0.3% phytagel (proliferation medium) [18], for the formation of axillary buds and development of shoots. The stock cultures were maintained by subculturing (every 4 weeks) the new formed shoots on fresh proliferation medium for further experiments. After the fourth subculture the light intensity was reduced from 40 to 20 $\mu E.m^{-2}.s^{-1}$.

2.4. Effect of explant density on multiplication

To examine the effect of the explant density on the formation of shoots, four explants densities (3, 4, 6 and 7 shoots) per 132 cm^2 were tested, *in vitro* in glass jar (370 ml capacity) containing 30 ml of proliferation medium. For each group of densities, ten explants (three replicates each) were put in culture and the number of neoformed shoots was recorded 4 weeks later.

2.5. Effect of explant size on multiplication

The effect of explant size on axillary bud formation (proliferation) was evaluated by culturing explants of different sizes (< 1 cm, 1 to 1.5 cm, 1.5 to 2 cm and > 2 cm) on the proliferation medium. Each experiment included three replicates with at least ten explants per replicate. For the proliferation experiments, the numbers of shoots that developed were recorded 1 month after the beginning of the experiment.

2.6. Statistical analysis

Data corresponding to the numbers of new formed shoots and the percentage of survivals (recovery) were collected after four weeks, and were analyzed using the analysis of variance (ANOVA). The differences among means (P=0.05) were compared using Duncan's test at 5% probability level.

3. Results

3.1. Culture establishment and shoot induction (proliferation)

The seeds were germinated in the establishment medium, containing 1 $mg.l^{-1}$ IBA and 0.1 $mg.l^{-1}$ GA_3. After 6 weeks of the *in vitro* culture, plantlets with 4–8 leaves developed. These plantlets were decapitated and cultivated on the proliferation medium, added with 1.0 $mg.l^{-1}$ kinetin and 0.1 $mg.l^{-1}$ NAA. Within 2 weeks of culture, we observed axillary buds that emerged from the axils of the leaves, which then developed into shoots. The axillary shoots produced from each explant were used to determine the parameters that affect the proliferation of globe artichoke.

3.2. Effect of explant density on multiplication

Our results showed that the density of the explants did have a clear effect on the proliferation of globe artichoke (figure 1). In fact, the reduction of this density from 6-7 to 3-4 explants per 132 cm^2 of growth area increased significantly the rate of multiplication from 2.33 to 6.57 shoots (figure 1A). As shown in figure 1B, the

percentage of explants survival was maximal for 3 to 4 explants per unit of the area and showed a significant decrease when this density was higher (> 6). This suggests that, at low density, the explants benefit from a better nutrition and a lower quality of exudates released by shoots.

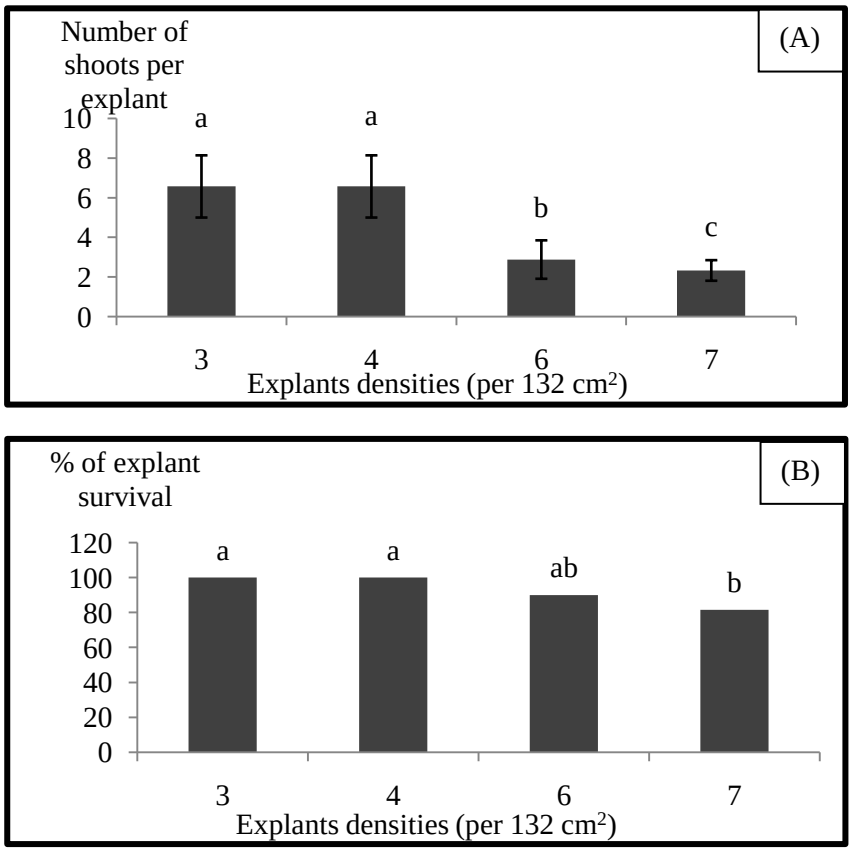

Figure 1: Effect of explant density on axillary bud proliferation (A) and percentage of explants survival (B) in globe artichoke explants cultured in proliferation medium for 4 weeks. Means followed by the same letter are not significantly different according to Duncan's test (P=0.05).

3.3. Effect of explant size on multiplication

The effect of explant size on the regeneration of the plant was established by culturing different sizes of explants in a proliferation medium. We observed that the size of the initial explants affects the rate of proliferation and the number of axillary shoots, under 1 cm of explant size, only 1.06 shoots per explants have grown, of which 26.6 % became necrotic after two weeks of culture. In the explants with a size between 1-1.5 cm and 1.5-2 cm, shoots production was significantly higher compared to the other sizes, reaching 7.73 and 7.30 shoots per explants respectively (figure 2A). By contrast, increasing the size of the explants, over 2 cm, results in the reduction of the number of shoots per explants to reach only 2.76. This might be the consequence of the apical dominance, which inhibits the formation of adventitious buds. For explants over 1cm of size, the percentages of survival in culture (73.4–100%) was not significantly affected (figure 2B).

4. Discussion

Our report is the first to describe the effect of the density and the size of explants of globe artichoke on shoots formation and proliferation. Even if the parameters related to the size and the density were relatively straight forward to test and undemanding to apply, they were unexpectedly ignored in previous studies. Indeed, most of the previous reports were focused on the optimization of the plant growth regulators and the composition of the medium.

We demonstrated here that a high number of shoots per glass jar reduced their growth. This is probably due to the competition for nutrients between shoots in the jar. This result obtained with globe artichoke is consistent with those obtained on poplar, which showed that the number of cultured shoots per vessel has a significant influence on shoot proliferation [19]. In addition, it was reported elsewhere that the surface area available to the

culture might, to some extent, regulates the number of axillary shoots that can develop [20]. We showed that densities comprised between three and four explants per culture jar gave similar results. However, the density of four explants could still be a better choice in order to reduce the cost of the big scale production. In support of this argument, it was recently shown that the cost of *in vitro* production of Smooth cayenne pineapple shoots could be halved by culturing at a density of 3 separated shoots per culture compared to explant density of one shoot per culture [21].

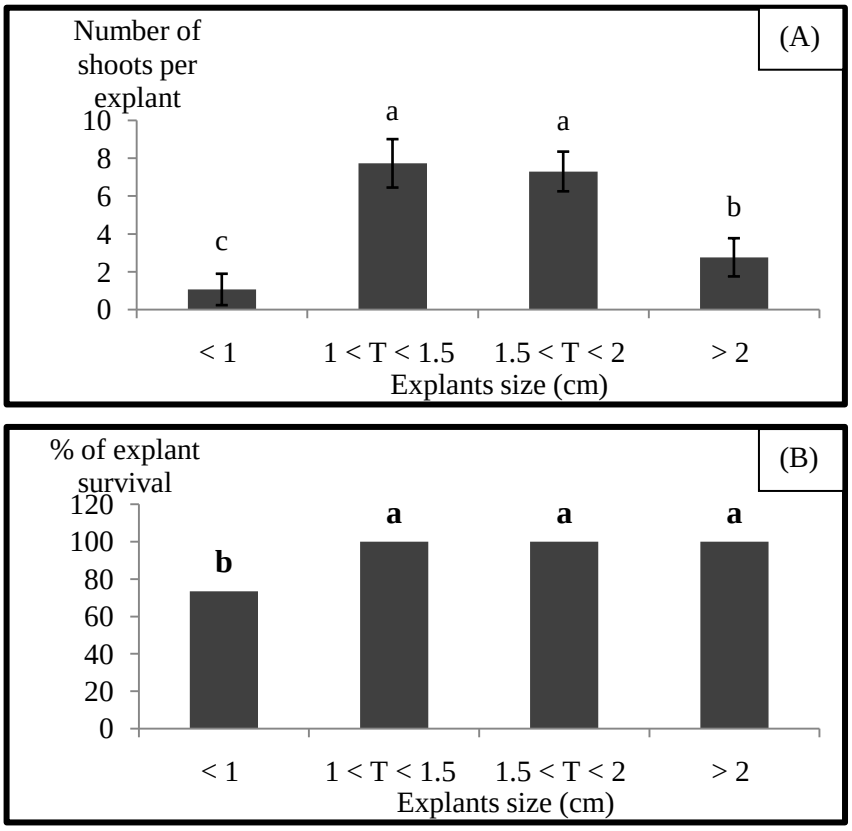

Figure 2: Effect of size on shoot proliferation (A) and percentage of explants survival (B) of globe artichoke explants cultured in proliferation medium for 1 month (T: Size). Means followed by the same letter are not significantly different according to Duncan's test (P=0.05).

In the present study, shoots with a size comprised between 1 and 1.5 cm, gave better proliferation rate compared to that of smaller or bigger sizes. The effect of the explants size on shoots formation was previously examined for others species, in particular gerbera [22] and cedar [23]. Elsewhere, it was suggested that the explants size has an effect on the response of the tissue [24]. This was supported by other reports which indicate that the explants size is an important factor affecting axillary bud proliferation in plant tissue culture [23]. Probably, the larger explants contain more nutrient reserves and endogenous plant growth regulators to sustain the culture [24]. This was confirmed by other reports showing that large explants generally survive more frequently and grow more rapidly at the outset than very small ones [25]. Accordingly, a positive correlation was shown between explants size and their age. Indeed, Nhut *et al*. (2007) [22] reported that if the bud age of gerbera increased, the regeneration ability significantly increased. These authors concluded that the development of shoots depend not only on the physiological state of the buds, but also depends on their size. According to the same authors, the size might play a more important role, because larger tissues had more nutrient reserves, which by consequence promote the development of shoots.

Conclusions

In this study, we examined the effect of explants density on the *in vitro* budding of globe artichoke and we determined the appropriate explants size for high shoots proliferation. These findings could be a useful tool to improve the rate of *in vitro* multiplication of globe artichoke and may contribute to promoting the development of *in vitro* culture of artichoke by reducing the waste of crops, culture media and growth factors.

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