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In Vitro Growth of Sunflower (*Helianthus annuus*) via Direct Organogenesis

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ABSTRACT

Sunflower (*Helianthus annuus*) is a crop of increasing importance as a source of seed oil and proteins; nonetheless the number of studies on sunflower tissue culture is somewhat limited. The development of a competent *in vitro* direct organogenesis protocol involves important basic steps of regeneration. In our study, chemically sterilized sunflower seeds were planted on induction media and 52.54 % germination efficiency was found. While the seeds were subjected to regeneration containing 2 mg/L of cytokinin, Benzyl Adenopurine (BAP) as well as 1 mg/L of auxin, Naphthalene Acetic Acid (NAA); shoot growth was observed with41 % regeneration efficiency. Non-sterilized seeds germinated but showed fungal growth on the surface of media resulting in no regeneration of sunflower plantlet. On the other hand, sterile seeds germinated less with little or no fungal growth leading to successful regeneration. Frequent regeneration of sterile sunflower seeds through direct organogenesis can contribute to enhanced micro-propagation of this plant.

Keywords: BAP, Cytokinin, Efficiency, Direct organogenesis, Explant, NAA, Regeneration, and Sunflower.

INTRODUCTION:

Sunflower, a Composite family member, is one of the major edible oil seed crops cultivating worldwide along with soybean, rapeseed and groundnut (Dagustu, 2018). Different factors include germplasm insufficiency, disease vulnerability, seed dormancy, recalcitrance to regeneration and abiotic factors cause the reduction of sunflower yield leading towards economic losses (Moghaddasi, 2011; Sujatha *et al*., 2012; Wingender *et al*., 1996). As there is limited use of native varieties due to genetic barriers in the reproduction process, conventional approaches fail to attain the desired growth and advancement of sunflower (Fiore *et al*., 1997; Gürel and Kazan, 1998; Shahen *et al*., 2019). Improved quality of sunflower plants can be obtained by gene transfer from wild species and other crops. A consistent, functional *in vitro* regeneration method followed by transformation is mandatory for efficient gene transfer in sunflower (Firoz *et al*., 2016; Khatun *et al*., 2016; Hewezi *et al*., 2003).

Successful direct organogenesis guided regeneration of sunflower is adaptive and depend upon several factors including age, plant ecotype, environmental conditions and composition of culture media (Alibert *et al*., 1994; Deglene *et al*., 1997; Sarrafi *et al*., 1996). Direct organogenesis, which is the regeneration of plantlet without callus induction phase, is required in modern breeding to ensure reduced cost and rapidity in regeneration (Pourhosseini *et al*., 2013). Tissue culture dependent

direct organogenesis from seeds have been done in various plants (Giovannelli *et al*., 2004; Zhu *et al*., 2007); but limited information has been reported regarding sunflower. Furthermore, sunflower tissue culture has been undertaken with different explants, including cotyledons, immature embryo, protoplast, shoot tips, hypocotyls (Fiore *et al*., 1997; Dagustu, 2018).

Use of seeds as explant in case of sunflower regeneration is barely reported. Hence, in our study; we have performed *in vitro* direct organogenesis from cultured seed of sunflower. Seedlings obtained by this technique can be further used for genetic modification with less chance of contamination.

MATERIALS AND METHODS:

Sunflower seeds were brought from Bangladesh Agricultural Development Corporation (BADC), Meherpur, Bangladesh. Healthy seeds were subjected to germination. Seeds were incubated at 56° C for 3 hours. After that, seeds were dehusked followed by sterilezation. Dehusked seeds, which were washed with tap water for 5 times, were rinsed with 70% alcohol for 2 minutes followed by 20 minutes soaking in 40% sodium hypochlorite with two drops of tween 20 with frequent stirring. Then the seeds were washed 5 times using autoclaved distilled water in order to ensure removal of disinfecting residues. At last, the seeds were dried onto sterile whatman filter paper in lamina airflow until dried. These disinfected dried seeds were placed to germinate on induction media containing phytagel (0.3%) under laminar airflow.

The sterilized seeds and the control (non-sterilized counterpart), were placed angularly in such manner that one-third of each seed dipped inside the media. The flask was sealed and placed in a light and temperature controlled plant growing system (constructed by Science Factory, DU) at 22°C and 16/8 h light/dark condition.After germination, seeds were transplanted to regeneration media (sucrose 3 %, macro-nutrients 5 %, micro-elements 0.5 %, myo-inositol 0.01 %, casein hydrolysate 0.05 %, BAP 2mg/L, NAA 1 mg/L; pH 5.8). On the course of regeneration, 8 days old plantlets were soaked in antifungal cefotaxime solution (200 mg/ml) for 15 minutes and then transplanted onto new regeneration media.

RESULTS AND DISCUSSION:

Seeds started to germinate from day two. Sterilized seeds took 4-5 days to germinate and grew slowly. Non-sterilized seeds took 1-2 days to germinate and growth rate was higher. At the 5th day of the germination, efficiency was recorded. Non-sterilized seeds indicated fungal growth on the upper surface of media after germination (not shown). However, sterile seeds showed lower germination (52.54 %) compared to those of non-sterile ones (70 %) but showed less fungal growth (**Table 1**).

A possible reason behind the reduction of germination of sterilized seeds could be the inability of persisting the perilous influence of the chemical sterilants. At the 8th day of germination, cefotaxime treated seedlings were shifted to regeneration. In the regeneration media the seedlings showed variability in growth pattern. At the 14th day of germination, final regeneration efficiency was calculated (**Table 1**). It was noticed that plantlets regenerated from sterile seeds showed 41 % regeneration efficiency and longer shoot (**Fig 1.c**). Higher shoot growth of sunflower was obtained due to elevated cytokinin-to-auxin ratio.

On the contrary non-sterilized germinated seeds failed to regenerate (**Table 1**) (**Fig 1.d**) since fungal contamination might occur from the seeds.In our study, sunflower culture was a challenge due to frequent fungal contamination. It was reported that cultivation of sunflower could face many constrains including microbial infection, precocious flowering, reduced rooting, hyperhydricity (Taški-Ajduković and Vasić, 2005). In spite of the challenges, we were successful to regenerate sunflower seedlings at *in vitro* conditions.

Table 1: Seed germination and regeneration

Seed Types	Total Seeds	Germinated Seeds	Germination Efficiency (%)	Regenerated Plant	Regeneration Efficiency (%)
Sterilized	118	62	52.54	48	41
Non-sterilized	50	35	70	0	0

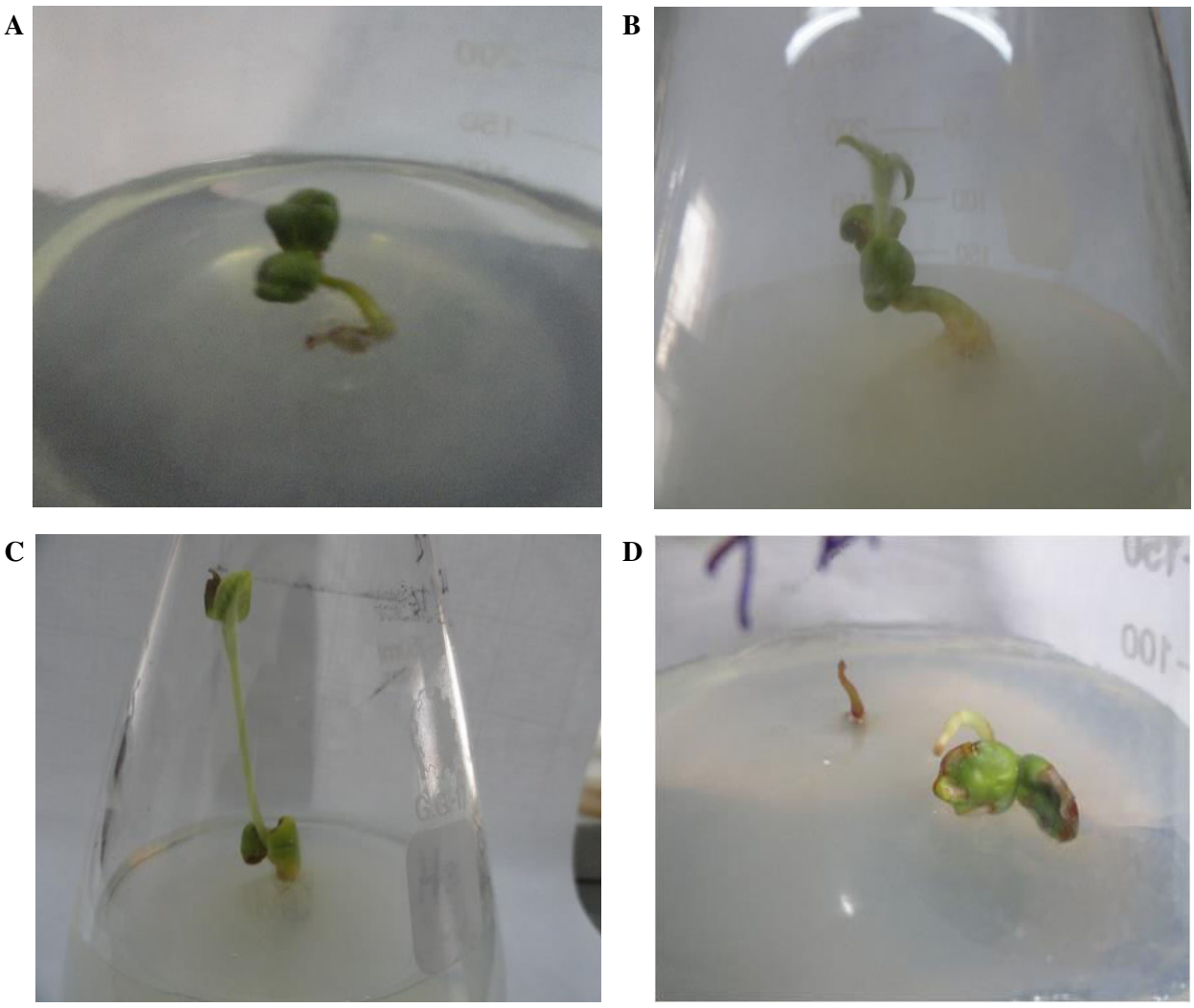

Fig1: Direct organogenesis of sunflower plants from seed explants. (A-C) Six, ten- and fourteen-days old plantlet of the germinated sterile seeds; (D) Germinated non-sterile seeds which failed to regenerate. Cytokine and auxin ratio during regeneration, BAP: NAA = 2:1.

CONCLUSION:

Direct organogenesis can increase the rapidity of plant regeneration and reduce the cost. *In vitro* direct organogenesis of sunflower using seed as explant can overcome environmental constrain. Also, sterile plantlets/plants can be developed by this method. Furthermore, these plantlets can be utilized for genetic transformation by particle bombardment or other biotechnological process and produced at large scale by micropropagation. Hence, the study we performed is important in plant biotechnology.

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CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

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