EFFECT OF FLORET WHORL AND CULTURE MEDIA ON ANTHER CULTURE IN SUNFLOWER

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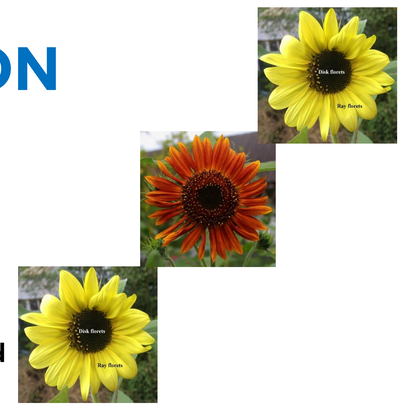

Abstract: The main aim of this research was to investigate the effect of floret whorl and culture media on haploid plant production via anther culture in sunflower. Three outermost unopened disk florets of the flower head at the R5.1 reproductive stage of three sunflower varieties including S473, Pacific 22 and Prado Red were used in this study. For haploid plant production via anther culture, anthers were cultured on various callus induction media for 30 days, and the anther-derived embryogenic calli were subsequently induced on shoot induction medium for 14 days. Then, the embryogenic calli were immerged in 100 and 300 µM of colchicine solution for 3 and 6 hours for chromosome doubling. The polyploidy level was determined with flow cytometry. It was found that callus growth parameters (callus size, fresh weight, and dry weight), the percentage of callus and the percentage of embryogenic calli were significantly affected by medium types. MS medium supplemented with 2 mg/l NAA, 1 mg/l BAP and 10% (v/v) CW induced the highest percentage of calli for all three varieties with the highest frequency of 65.48%. For shoot induction study, embryogenic calli gave the best response on MS medium supplemented with 2 mg/l BAP, 500 mg/l CH and 0.2% activated charcoal. Some embryogenic calli could develop into shoot or root but not a complete plant. Colchicine concentrations and treatment durations had a significant effect on the survival rate and growth of callus. Optimization of anther culture with regard to high efficiency shoot regeneration are needed to be further investigated.

Introduction

Anther culture is one of the very popular methods for production of haploids through culturing anthers or microspores on artificial culture medium. It allows novel allele combinations, particularly ones involving recessive characters, to be assessed in intact plants. The haploid duplicates the chromosome complement in order to obtain homozygous diploids (Baenziger and DePauw, 2009). In pollen derived plants, duplication of chromosomes may occur spontaneously in cultures, but due to the small percentage of such double-haploids, it is necessary to double the haploids by colchicine treatment (Fig. 1). Haploid induction through anther culture depends on many factors such as genotype, physiology of the donor plant, culture medium, culture density, microspore developmental stage and environment of culture condition (Bhojwani and Bhatnagar, 2009).

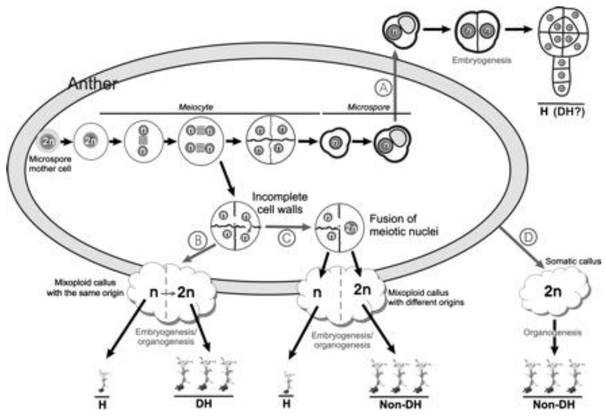

Fig. 1 Diagram showing the different in vitro developmental alternative pathways of anther culture (Seguí-Simarro and Nuez, 2006)

Objective

To investigate the effect of floret whorl and culture medium on anther culture of three sunflower varieties

Materials and Methods

Flower buds at R5.1 stage of S473 (synthetic), Pacific 22 (hybrid) and Prado Red (hybrid) varieties were sterilized and their anthers were carefully removed from the three outermost whorls of disk florets, namely 1, 2 and 3 (Fig. 2). Anthers were cultured on callus induction medium (A1-A4) and subsequently sub-cultured on shoot induction medium (S1-S4) (**Table 1**). The embryonic calli or active calli were selected for chromosome duplication study using colchicine solution at various concentrations 0 µM (control), 100 µM and 300 µM and incubated for 3 h and 6 h in dark condition.

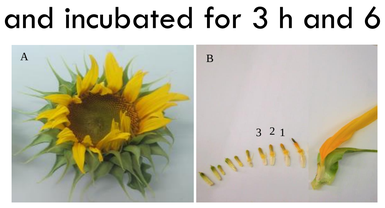

Fig. 2 Images of flower head at R5.1 reproductive stage (A) and disk florets (B).

Table 1. Callus induction medium (A1-A4) and shoot induction medium (S1-S4) for anther culture study.

Media	Basal	CH	CW	Auxin (mg/l)		Cytokinin (mg/l)
		(mg/l)	(ml/l)	NAA	2,4-D	BAP
A1	MS	500	-	2	-	1
A2	MS	-	100	2	-	1
A3	MS	500	-	-	0.5	0.5
A4	MS	-	100	-	0.5	0.5

Media	Basal	CH (mg/ml)	$AgNO_3$ (mg/ml)	Charcoal (%)	BAP (mg/l)
S1	MS	500	2	-	1
S2	MS	500	2	-	2
S3	MS	500	-	0.2	1
S4	MS	500	-	0.2	2

Selected References

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Todorova, M., Ivanov, P., Shindrova, P., Christov, M., and Ivanova, I. (1997). Double haploid production of sunflower (*Helianthus annuus* L.) through irradiated pollen-induced parthenogenesis. Euphytica. 97: 249-254.

Results and Discussion

Analysis of variance for different floret whorls was significant in the percentage of callus induction and the percentage of embryogenic callus parameters. The interaction of whorl x medium was significant in callus size, the percentage of callus induction and the percentage of embryogenic callus. MS medium supplemented with 2 mg/l NAA, 1 mg/l BAP and 10% (v/v) CW induced the highest percentage of calli for all three sunflower varieties with 65.48%. Analysis of variance confirmed that callus growth (dry weight), percentage of anther-derived embryos and percentage of callus were significantly affected by medium and floret whorls. For shoot induction study of anther-derived embryogenic calli, MS medium supplemented with 2 mg/l BAP and 0.2% activated charcoal was found to be most suitable for shoot regeneration (1.85%) and root formation (1.94%) compared to other medium which only embryogenic calli were produced. Embryogenic calli treated with 100 µM and 300 µM of colchicine gave the survival rate about 93.20% and 82.52%, respectively. In addition, callus size, fresh weight, and dry weight of treated calli were decreased in colchicine treatment compared with control treatment. No regeneration was observed when colchicine was added after three weeks of treatment. The results of this study were in agreement with previous studies in which various genotypes and medium composition showed differential responsiveness towards callus initiation and haploid plant production (Gurel et al., 1991; Todorova et al.,1997; Saji and Sujatha, 1998; Priya et al., 2003; Dayan, 2016).

Factors	CS (mm)	FC (mg)	DC (mg)	PC (%)	PE (%)
Variety					
S473	2.019±1.069	0.142±0.088	0.050±0.032a	42.28±33.88	9.63±8.77
Prado Red	1.897±1.131	0.148±0.089	0.051±0.032ab	36.42±30.08	10.37±9.27
Pacific 22	2.036±1.233	0.157±0.087	0.058±0.032b	38.74±27.03	9.70±9.67
F-test	ns	ns	**	ns	ns
Whorl					
1	1.974±1.228	0.148±0.091	0.055±0.035a	45.03±33.66b	10.48±9.63a
2	1.985±1.232	0.147±0.088	0.050±0.032a	37.69±61.02a	11.15±10.17b
3	1.981±0.962	0.153±0.087	0.053±0.039b	31.72±24.75a	8.07±7.44a
F-test	ns	ns	**	**	**
Medium					
A1	2.584±0.325c	0.197±0.023c	0.067±0.010b	58.70±20.99c	12.69±20.99c
A2	2.828±0.517c	0.214±0.021c	0.079±0.012c	65.48±22.19c	21.88±22.19d
A3	2.053±0.791b	0.172±0.042b	0.058±0.016b	27.56±17.72b	5.04±17.72b
A4	0.00a	0.00a	0.00a	0.00a	0.00a
F-test	**	**	**	**	**

Table 2 Comparison of the mean values (± SD) of callus induction characteristics in three sunflower varieties.

Remark: Means followed by the same letters are not significantly different at p<0.01 level of probability using DMRT.
** = Significant at 0.01 probability level.
CS = callus size, DC = dry weight, FC = fresh weight, PC= the percentage of callus induction, and PE = the percentage of embryogenic calli.

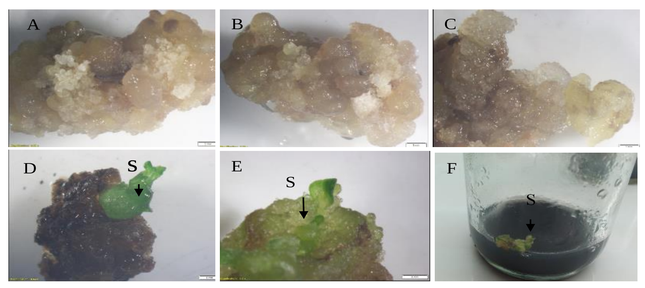

Fig. 3 Embryogenic callus (ES) and shoot (S) formation on induction medium in sunflower. A) ES of Pacific 22, B) ES of S473, C) ES of Prado Red, D) Shoot of S473, E) Shoot of Pacific 22, and F) Shoot of Prado Red.

Conclusions

Factors influencing anther culture response were investigated in this study. Callus growth (dry weight), the percentage of callus and the percentage of embryogenic calli were significantly affected by medium and floret whorls. MS medium supplemented with 2 mg/l BAP and 0.2% activated charcoal was found to be most suitable for shoot regeneration and root formation compared to other tested media. Survival rate, shoot and root production of regenerating calli following in vitro colchicine treatment decreased with increasing colchicine concentration and treatment time. No doubled haploid plants were regenerated from calli treated with colchicine. Future research should focus on the shoot induction through addition of other plant hormones, additives or inhibitors since shoot production from sunflower plants is very low and plant regeneration is problematic.

Acknowledgement

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