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BRACT AS AN EXPLANT FOR CALLUS INDUCTION AND SHOOT BUD FORMATION IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

Tender bracts were used for callus induction and shoot bud formation in "KBSH-1", "BSH-1" and "Morden" genotypes of sunflower. High frequency callus induction was observed on MS medium supplemented with NAA or 2, 4-D alone or in combination with BA. Such callus-regenerated shoot buds after four weeks of incubation on MS medium with 1.0 mg/l BA in "KBSH-1" only. Further studies with other genotypes, different media compositions and culture conditions are needed to obtain high frequency of regeneration.

Key words : Sunflower, bract explant, callus induction, shoot bud formation.

INTRODUCTION

Sunflower is one of the world's most important oilseed crops. Maintaining sunflower as a competitive oilseed crop depends largely on its continued improvement to meet changing of cultivational conditions. The application of biotechnological methods for sunflower improvements is mainly limited by the difficulty of regenerating plants in a reproducible and efficient fashion. Plant regeneration has been reported from a variety of tissues including immature embryos (Espinasse et al., 1991), hypocotyls (Lupi et al., 1987), cotyledon (Ceriani et al., 1992), and even from protoplasts (Trabace et al.,1995). However, these results are restricted to a few genotypes under specific conditions. So far, the only explant allowing reproducible plant regeneration in many cases is immature embryo (Knittel et al., 1991).

Keeping in mind the explant specificity in morphogenic response, we report here that young bracts can also be used as an explant for callus induction and plant regeneration, thus providing an additional source of explant material.

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MATERIALS AND METHODS

The present experiment included three sunflower genotypes viz., "BSH-1" and "KBSH-1" hybrids and "Morden", an open-pollinated variety. Bracts obtained from capitula (2.0-2.5 cm diameter) of field grown plants were surface sterilized with 0.2% (w/v) mercuric chloride. After removing the peripheral bracts, the innermost, tender bracts were cut into 2-3 pieces and cultured on Murashige and Skoog (1962) agar medium at pH 5.6-5.8, supplemented with different auxins and cytokinins at different levels and sucrose (30 g/l). The cultures were incubated at 25 ± 2°C under 12 h photoperiod with light intensity of about 1000 lux.

RESULTS AND DISCUSSION

Callus induction

Bract explant expanded initially and callus was formed from cut ends, within a week in majority of the explants. All the explants cultured produced callus (100%) on MS medium containing 1.0 mg NAA or 2.0 mg 2,4-D or 1.0 mg/l each of NAA and BA in all three genotypes (Table 1). In "KBSH-1", medium with 1.0 mg/l 2,4-D also resulted in 100% callus induction. Callus induction from any explant in sunflower is relatively easy. Auxins alone or in combination with cytokinins result in callus induction (Lupi et al., 1987; Ceriani et al., 1992) in most explants of sunflower. Even BA at 0.5 mg/l resulted in callus induction at low frequency (21.2%). Similar effect of BA on callus induction from other explants of sunflower (Greco et al., 1984; Lupi et al., 1987) has been reported.

Table 1: Effect of different growth regulators on callus induction from bract explants of sunflower genotypes.

Growth regulators (mgl^{-1})	Per cent callus induction*			Mean (%) ±2.38
	KBSH-1	BSH-1	Morden	
NAA (1.0)	100.0	100.0	100.0	100.0 (90.00)
NAA (0.5) + BA (1.0)	88.5	92.0	97.2	92.5 (76.77)
NAA (1.0) + BA (1.0)	100.0	100.0	100.0	100.0 (90.00)
2,4-D (1.0)	100.0	88.5	86.5	91.6 (76.55)
2,4-D (2.0)	100.0	100.0	100.0	100.0 (90.00)
2,4-D (2.0)+BA(1.0)	89.6	90.9	92.8	91.1 (77.96)
BA (0.5)	25.9	10.2	27.7	21.2 (26.92)
Mean (%) ±1.56	86.2 (76.80)	83 (72.24)	86.3 (75.00)	85.2 (74.73)

Average of two replications.
Figures in parenthesis and SE are arc sine values.

It has been speculated that there might be enhancement of the level of one growth regulator by the other leading to promotion of biosynthesis or inhibition of degradative metabolism (Gressoff, 1978) or due to the effect of BA itself on dedifferentiation (Greco et al., 1984). The fact that low levels of both auxins and cytokinins initiate callus formation, on their own or in combination, itself is a problem as it leaves very little room for manipulation of auxin-cytokinin balance for plant regeneration.

Callus produced in the study was compact, globular and white on medium containing 0.5 mg or 1.0 mg NAA and 1.0 mg/l BA but it was loose and translucent on other growth regulator combinations. Direct root formation from explants of 'Morden' was found on medium with 1.0 mg/l of NAA and BA.

Morphogenesis

Callus derived on 0.5 mg/l BA, regenerated into shoot buds in five percent of "KBSH-1" cultures after four weeks incubation on medium containing 1.0 mg/l BA. Root morphogenesis was also noticed from parts of the callus. Calli from "BSH-1" and "Morden" turned into hard, compact and white nodules but did not undergo shoot or root morphogenesis. BA was found to be essential for shoot bud formation. Cytokinin-induced shoot bud formation has been reported by Greco et al., (1984) and Lupi et al., (1987) in sunflower. The response from bract callus was restricted to "KBSH-1", indicating genotype specific cytokinin effect on organogenesis. In order to achieve high frequency of regeneration, further studies with other genotypes, different media and culture conditions are needed.

Probably this is the only study which shows that young bracts can be used as source of explants for producing morphogenic callus in sunflower.

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LAS BRACTEAS COMO EXPLANTES PARA INDUCCIÓN DE CALLO Y FORMACIÓN DE TALLO Y DE YEMAS EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

Bracteas tiernas fueron usadas para la inducción de callo y formación de tallos y yemas en genotipos de girasol "KBSH-1", "BSH-1" y "Morden". La alta frecuencia de inducción de callo fue observada en medio MS suplementada con NAA o 2-4-D solo o su combinación con BA. Este callo regeneró yemas de tallo después de Posteriores estudios con otros genotipos, diferentes composiciones de medio y condiciones de cultivo son necesarios para obtener alta frecuencia de regeneración.

L'UTILISATION DES BRACTÉES COMME EXPLANT POUR L'INDUCTION DE CALS ET DE BOURGEONS CHEZ LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

De jeunes bractées ont été utilisées pour induire la formation de cals et de pousses chez les génotypes de tournesol "KBSH-1", "BSH-1" et "Morden". Une fréquence élevée d'induction callogène a été observée sur le milieu MS complété avec NAA ou 2,4-D seuls ou en combinaison avec BA. Ces cals ont régénéré des pousses après 4 semaines d'incubation sur le milieu MS additionné de 1.0 mg/l de BA seulement chez "KBSH-1". Des études complémentaires sur d'autres génotypes, sur différentes compositions de milieux et conditions de culture sont nécessaires pour obtenir des fréquences élevées de régénération.