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Plant regeneration *in vitro* in different cultivars of sesame (*Sesamum indicum* L.)

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Abstract. Tissue cultures of 7 cultivars of sesame (*Sesamum indicum* L.) were established using seedling explants. Multiple shoot buds were observed in shoot tip cultures in all the cultivars with different frequencies. Shoot tips excised from seeds pretreated with cytokinin increased the frequency of induction of multiple shoot buds. Rooted plantlets from isolated shoot buds were successfully established in soil. The plants have matured and flowered.

Keywords. *Sesamum indicum*; cytokinin; multiple shoot buds; plantlet regeneration.

1. Introduction

Sesame (*Sesamum indicum* L.) is an important edible oil crop cultivated in several parts of the world. In India, about 2–3 million hectares of land are utilized for the cultivation of sesame alone. However, its cultivation is very much restricted to poor soils due to several constrains such as low and unreliable yield, shattering, high production cost and low return to the farmers (Murthy *et al* 1985). In order to develop varieties possessing higher and more stable yields and adapted to different climatic conditions sesame breeding based on conventional methods has been in progress during the last several decades. To supplement these efforts, tissue culture of sesame was initiated because somaclonal variants obtained *in vitro* have been successfully used in breeding programmes to develop new genotypes in crop plants (Larkin *et al* 1985). In an earlier report (George *et al* 1987) we have described a technique for *in vitro* propagation for sesame through induction of multiple buds in shoot tip cultures. The present communication describes steps in the *in vitro* multiplication of different cultivars of sesame.

2. Materials and methods

Seeds of cultivars PT, N8, N128, N62-32, TC-25, T-12 and 'Hawary' (obtained from Nuclear Agriculture Division, Bhabha Atomic Research Centre, Bombay) were used as the source material. About 100 seeds of each cultivar were tied in a muslin cloth and surface sterilized with 0·1% $HgCl_2$ solution for 4–5 min. After repeated washing with sterile distilled water, the seeds were soaked in 6-benzylaminopurine (BA 8 mg/l) for 72 h and then germinated on solid MS medium (Murashige and Skoog 1962) containing BA (8 mg/l). For control the seeds were soaked in water for 72 h. Similarly seeds were also pretreated with zaetin (Z) and 6-(γ,γ-dimethylallylamino) purine (2,iP, 8 mg/l) to study the effect of these cytokinins on multiple bud induction in the various cultivars. Shoot tips with cotyledons were excised from 10–12 days old seedlings and cultured on MS basal medium with BA (8 mg/l). After 6

weeks of culture, multiple adventitious buds induced in shoot tips were transferred to MS medium containing BA (1 mg/l) for further growth. The shoots were thereafter isolated and transferred to 1/2 strength MS with 0·1 mg/l NAA for rooting. The rooted plantlets were first transferred to sterilized soil in paper cups and eventually established in the field.

3. Results and discussion

3.1 *Germination of seeds*

The per cent germination of non-soaked seeds was above 90% for all the cultivars except 'Hawary' in which it was about 70%. Pre-soaking of the seeds in a cytokinin medium reduced the percentage of germination in all the treatments.

3.2 *Culture of shoot tips with cotyledons*

Shoot tips with cotyledons excised from non-soaked seeds were cultured on MS basal medium containing BA, 2, iP or Z (8 mg/l). Cultivars T-12 and PT produced multiple buds in low frequency (20–40%) in all the 3 cytokinins while the response of the other cultivars was almost negative (10–20%).

Shoot tips with cotyledons excised from seeds pretreated with cytokinin showed increased frequencies of multiple buds. Of the cytokinins, BA (8 mg/l) was most favourable for multiple bud formation in all the cultivars. Among the 7 cultivars tested, 'Hawary' showed the best response in 80% cultures producing multiple buds ranging from 15–18 per explant (figure 1A). In TC-25 it was 44% with 4–5 buds per explant. In other cultivars, the frequency of multiple bud induction varied from 58–70% with 6–15 buds per explant.

Shoot tip explants isolated from seeds pretreated with Z showed a low tendency for multiple bud induction. In cultivars PT, 'Hawary' and T12 multiple buds were produced in 50% of cultures whereas in N8 it was 33% and in N128, TC25 and

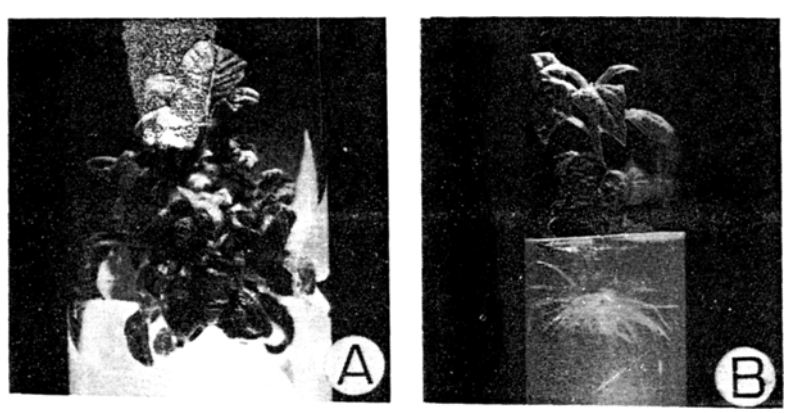

Figure 1. Plant regeneration *in vitro* in different cultivars of sesame. **A.** Multiple bud induction in cultivar 'Hawary' (MS + BA 8 mg/l). **B.** Rooting of an excised shoot.

Table 1. Response of shoot tips of different cultivars of sesame to shoot bud regeneration on MS + BA, MS + Z (8 mg/l).

	Cultures showing multiple bud formation		No. of shoot buds regenerated per explant	
Cultivar	BA (%)	Z (%)	BA	Z
T-12	58	52	8–10	4–6
TC-25	44	11	4–5	4–5
PT	70	56	12–15	10–12
Hawary	80	53	15–18	8–10
N 8	63	33	6–10	5–6
N 128	58	18	12–15	3–4
N 62–32	65	20	10–12	5–8

N62–32 it was less than 20%. However, the number of buds per explant was less than in BA medium and it varied from 4–12 for different cultivars.

The cytokinin 2, iP (8 mg/l) was the least favourable for multiple bud formation. Among the cultivars tested PT (50%) and N62–32 (46%) showed better response. Others showed only one or two cultures having multiple buds. The number of buds per explant varied from 6–10 in PT and 4–6 in N62–32 (table 1).

The multiple buds when transferred to MS medium containing a lower concentration of BA, Z or 2, iP (0·5 or 1 mg/l) grew vigorously. All *in vitro* developed shoots were rooted in half strength MS medium with NAA (0·1 mg/l) (figure 1B). Rooted plants were transferred to paper cups and were established in soil where they flowered and set fruits.

The present study has demonstrated that in sesame, shoot tip culture of seedlings is a reliable method for *in vitro* propagation of different cultivars although the genotype of the donor parent influenced the response of the explant in culture as in other species (Frasekes and Palmer 1986).

Experiments are underway to grow large number of *in vitro* regenerants in the field for the evaluation of various agronomic characters. A few lines of regenerants have been carried forward to the second generation in the field.

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