Effect of genotype on shoot regeneration from cotyledonary explants of rapeseed (Brassica napus L.)

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Summary. Ability of shoot regeneration from cotyledonary explants of rapeseed (B. napus) was surveyed for 100 cultivars. Effects of explant age and plant growth regulators were determined before screening the genotypes. The optimal condition required 4-day-old cotyledons as explant and 4.0 mg/l benzylaminopurine as plant growth regulator. Gas-permeable tape as sealing material was more effective for shoot regeneration than Parafilm. When testing cultivars, shoot regeneration response was strongly influenced by genotype with a range of variation from 97% (percentage of explants regenerating shoots) in 'Arabella' and 'Norin 26' to 0% in 'Norin 18' and 'Norin 30'. The response was not dependent on origin and cropping type (spring vs. winter type). The ability of shoot regeneration was not related to that of microspore embryogenesis. The regenerants rooted on medium containing 2.0 mg/l indole-3-butyric acid and after planting in soil flowered and set seeds. Histological studies showed that shoot meristems developed in callus which had grown from the vascular bundle tissue within 8 days.

Key words: Explant genotype - Cytokinins - Benzylaminopurine - Organogenesis - Tissue culture

Abbreviations: BA: 6-benzylaminopurine; IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

Introduction

Rapeseed (Brassica napus) is an important oilseed crop worldwide. Many applications of tissue culture (Stringham 1977; Keller and Armstrong 1978; Dunwell 1981; Glimelius 1984) and genetic engineering (Pua et al. 1987; Fry et al. 1987; Neuhaus et al. 1987; Radke et al. 1988; Moloney et al. 1989) techniques have been used to improve this crop. Although B. napus is considered to be amenable to various kinds of tissue culture and transformation techniques, almost all reports to date are concerned with a limited number of genotypes only. On the other hand, intraspecific genotypic variation of regeneration ability have been reported in many species such as rice (Abe and Futsuhara 1984), alfalfa (Brown and Atanassov 1985), soybean (Komatsuda and Ohyama 1988) and barley (Taniguchi et al. 1991), and genetic factors regulating morphogenesis or regeneration have been analyzed (Bullock et al. 1982; Komatsuda et al. 1989).

In the present study, we report genotypic variation in the morphogenesis response in cotyledonary explants of a wide range of B. napus cultivars. In addition, attention is given to factors influencing morphogenesis and to histological development of the shoots.

Materials and Methods

Seeds of 100 cultivars of rapeseed, which are listed in Fig. 1, were kindly provided by Y. Okuyama and M. Ishida, Tohoku Nat. Agr. Exp. Sta., Morioka and T. Sakai, Planitech Res. Inst, Yokohama. Induction of shoot regeneration from cotyledonary explants was carried out according to Moloney et al. (1989) with minor modifications. Seeds were sterilized in sodium hypochlorite (1.0% active chlorite) with 1-2 drops of Tween-20 for 20 min. After rinsing in sterile distilled water three times, the seeds were placed in 15x90mm petri dishes containing plant growth regulator free MS agar (0.7%) medium (Murashige and Skoog 1962) at a density of 20 seeds per dish. Cotyledons including 1-2mm petioles were excised from 4-6 day-old seedlings. The petioles were embedded in regeneration media which were composed of MS basal medium supplemented with a combination of NAA (0-2.0 mg/l) and BA (0-8.0 mg/l) (see Fig. 2). Ten explants were cultured per 15x90mm petri dish. After 3 weeks of culture, adventitious shoots formed on the explants were counted. Regeneration frequency (number of explants with shoots / total number of explants) was averaged for at least 3 replications. Shoots induced in cotyledonary explants were transferred to MS agar medium containing various concentrations of IBA and NAA or lacking plant growth regulators for development of
the root system. All cultures were incubated at 25°C in a 16 h/day photoperiod of cool white illumination at 30μEms⁻¹. Regenerated plants were grown in vermiculite for about 2 weeks before transfer to soil in a greenhouse.

In order to study shoot development in petioles, cotyledonary explants of cv. Arabella, which were cultured on MS medium supplemented with 4.0 mg/l BA, were fixed in FAA after 0, 2, 4, 6, 8, 10 and 12 days of culture. Following dehydration in ethanol, the material was embedded in paraffin, was sectioned to 10μm in thickness and stained with hematoxyline (McManus and Mowry 1964).

Results and Discussion

Determination of optimal culture conditions for shoot regeneration

The effect of plant growth regulator concentration on shoot regeneration was tested with 12 combinations of BA and NAA using B. napus cv. Westar as shown in Fig. 2. The critical factor for shoot regeneration was the presence of BA in the medium. The maximum frequency of shoot regeneration (70%) was obtained in the presence of 4.0 mg/l BA. This agrees with a previous report by Moloney et al. (1989). In the absence of BA from the medium, no induction of shoot regeneration was observed. These results were also obtained with another cultivar of 'Arabella' (data not shown). The important role of BA for shoot differentiation in Brassica cotyledons has been reported previously (Moloney et al. 1989; Sharma et al. 1990; Hachey et al. 1991). On the other hand, Hachey et al. (1991) and Takasaki et al. (1993) described a requirement of NAA for the shoot regeneration from B. campestris cotyledons. Our results indicated that the addition of NAA decreased the frequency of shoot regeneration, but increased callus and root formation (data not shown), are consistent with results for B. napus.

Fig. 1. Regeneration of shoots from cotyledon explants of 100 cultivars of B. napus. Four-day-old explants were cultured on MS medium supplemented with 4.0 mg/l BA. Data consist of 3 replicates and for each replicate 10 cotyledon explants were used. Bar represents S.E. of the means. N: Norin, ■: Winter type, ▲: Spring type.

Fig. 2. Regeneration of shoots from 4-day-old cotyledon explants of B. napus cv. Westar on various concentrations of BA and NAA. Data consist of 3 replicates and for each replicate 10 cotyledon explants were used. Bar represents S.E. of the means.