Arunachalam, V., Narasimhulu, S. B., Shyam Prakash and Chopra, V. L. Comparative Shoot Regeneration in Diploid and Amphidiploid Brassica Species and their Interspecific Hybrids. *Canadian Journal of Botany*, 70 (1992): 1513-1514.

NOTE

Comparative shoot regeneration in diploid and amphidiploid *Brassica* species and their interspecific hybrids

S. B. NARASIMHULU, SHYAM PRAKASH, AND V. L. CHOPRA
Biotechnology Centre, Indian Agricultural Research Institute, New Delhi, 110 012, India

AND

V. ARUNACHALAM[1]
Division of Genetics, Indian Agricultural Research Institute, New Delhi, 110 012, India

Received November 10, 1991

NARASIMHULU, S. B, PRAKASH, S., CHOPRA, V. L., and ARUNACHALAM, V. 1992. Comparative shoot regeneration in diploid and amphidiploid *Brassica* species and their interspecific hybrids. Can. J. Bot. 70: 1513–1514.

Shoot regeneration response in interspecific hybrids of *Brassica* species were assessed in relation to the diploid pollen parents and amphidiploid female parents. Superior regeneration responses were observed in interspecific combinations of *B. carinata* and *B. nigra* (BBC), *B. juncea* and *B. campestris* (AAB), and *B. napus* and *B. campestris* (AAC). Though synthetic *B. napus* regenerated with a frequency less than that of the better regenerating parent (*B. oleracea*), higher regeneration response was observed in the hybrid between *B. napus* and *B. campestris*. Two triploid combinations of the genetic constitution ABC, one obtained by crossing synthetic *B. napus* with *B. nigra* and the other by crossing natural *B. juncea* with *B. oleracea*, showed low regeneration responses. The response improved substantially in a tetraploid of the constitution ABBC obtained by crossing *B. juncea* with *B. carinata*.

Key words: *Brassica*, alloploids, shoot regeneration.

NARASIMHULU, S. B, PRAKASH, S., CHOPRA, V. L., et ARUNACHALAM, V. 1992. Comparative shoot regeneration in diploid and amphidiploid *Brassica* species and their interspecific hybrids. Can. J. Bot. 70 : 1513–1514.

Les auteurs ont évalué la regénération des tiges chez des hybrides interspécifiques de *Brassica* spp. en relation avec les parents diploïdes polliniques et les parents femelles amphiploïdes. On observe des degrés de regénération supérieurs chez les combinaisons interspécifiques de *B. carinata* avec *B. nigra* (BBC), de *B. juncea* avec *B. campestris* (AAB) et de *B. napus* avec *B. campestris* (AAC). Bien que la fréquence de regénération du *B. napus* synthétique soit inférieure à celle des meilleurs parents (*B. oleracea*), on observe une regénération plus forte chez l'hybride entre *B. napus* et *B. campestris*. Deux combinaisons triploïdes de constitution ABC, une obtenue par croisement de *B. napus* synthétique avec *B. nigra* et une autre par croisement du *B. juncea* naturel avec *B. oleracea*, montrent des fréquences de regénération plus faibles. On observe une augmentation marquée de la fréquence de regénération chez un tétraploïde de constitution ABBC obtenu par croisement de *B. juncea* avec *B. carinata*.

Mots clés : *Brassica*, alloploïdes, regénération des tiges.

[Traduit par la rédaction]

Introduction

Intra- and inter-specific differences for shoot regeneration, observed in several crop plants, have established the genetic basis for regeneration response (Bogani *et al.* 1985; Fazekas *et al.* 1986). Such a genetic control has been exploited for breeding genotypes responsive to tissue culture (Bingham *et al.* 1975). The genetics of shoot regeneration is, however, not fully understood despite several attempts to characterize it (Buiatti *et al.* 1974; Frankenberger *et al.* 1981; Caligari *et al.* 1987). The genus *Brassica*, which contains diploid species *B. campestris*, *B. nigra*, and *B. oleracea* (genomes A, B, and C, respectively) and their alloploid combinations provides good material for studying interactive effects on shoot regeneration in crosses made with specific genotypes from each diploid species.

Species specificity for regeneration in *Brassica* has been inferred from comparative responses of diploids and natural amphidiploids (Dietert *et al.* 1982; Murata and Orton 1987) and diploids and amphidiploids synthesized from known diploids (Narasimhulu *et al.* 1988). The present study analyses shoot regeneration, i.e., organogenesis, response in cotyledon explants cultured *in vitro* to gain a further understanding of genome interactions in interspecific hybrids.

Materials and methods

Seeds of parental *Brassica* species and their hybrid combinations (Table 1) were surface sterilized with 0.1% mercuric chloride for 8 min, washed, and aseptically germinated in test tubes (15 × 2.5 cm) on moist filter papers at 25 ± 1°C on a 16 h light : 8 h dark cycle. Cotyledons with petioles, measuring approximately 0.4 cm, were excised from 6-day-old seedlings and inoculated on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with indole-3-acetic acid (IAA) at 2 mg/L and 6-benzyl amino purine (BAP) at 4 mg/L. The medium used was chosen on the basis of superior response after ranking regeneration responses of three diploid parental species and their four synthetic amphidiploid combinations in 10 different media containing a wide range of growth regulators (Narasimhulu *et al.* 1988). The medium contained 2% sucrose and was solidified with 0.8% agar after adjusting the pH to 5.8. In each experiment, 30–58 cotyledons were inoculated for every *Brassica* genotype with two cotyledons per culture tube (15 × 2.5 cm) containing 15 mL slanted agar medium. The cultures were incubated under cool white fluorescent light of 2500 lx intensity with a photoperiod of 16 h light : 8 h dark at 25 ± 1°C. The experiment was con-

[1]Author to whom correspondence should be addressed.

Printed in Canada / Imprimé au Canada

TABLE 1. Regeneration responses of *Brassica* species and their interspecific hybrids on MS medium supplemented with IAA (2 mg/L) and BAP (4 mg/L)

Species	Genomic formula	Mean regeneration (%)
Diploid (male parent)		
B. campestris spp. *oleifera* var. *brown sarson* cv. Pusa Kalyani (Ap)	AA	12.6
B. nigra cv. IC 257 (Bi)	BB	48.8
B. oleracea Var. *italica* (broccoli, Cb)	CC	25.1
B. oleracea Var. *botrytis* cv. Pusa Katki (Cp)	CC	39.6
Amphidiploid (female parent)		
B. juncea cv. Varuna (ABv)	AABB	38.5
Synthetic *B. napus* (ACs)	AACC	21.2*
B. carinata, acc. No. 1 (BC1)	BBCC	10.0
Triploid (hybrid)		
BC1 × Bi	BBC	65.9*†
ABv × Ap	AAB	48.2*†
ACs × Ap	AAC	27.0†
ACs × Bi	ABC	8.3*†
ABv × Cb	ABC	7.8*†
Tetraploid (hybrid)		
ABv × BC1	ABBC	34.8†

NOTE: Abbreviations in parentheses indicate species and genotypes. ACs is a colchicine diploidized product of Ap × Cp.
*Significantly different at $P = 0.05$ from better parent.
†Significantly different at $P = 0.05$ from mid-parent.

ducted three times. Regeneration percentages were compared at the end of the 4th week.

Data on percent shoot regeneration were subjected to arcsine transformation and analysed as a randomized design with three repeats (Gomez and Gomez 1976). Using transformed means, differences in regeneration percentage between a hybrid and its mid-parent (mean regeneration response of the two parental genotypes) or better parent (superior regenerating parent of the two parental genotypes) were tested by *t*-test at 5% level of significance; test of significance was performed on transformed values.

Results and discussion

Shoot regeneration of diploid species, which were the pollen parents for producing sesquidiploids, ranged from 12.6% for cv. Pusa Kalyani of *B. campestris* to 48.8% for cv. IC 257 belonging to *B. nigra* (Table 1). The differences in regeneration potential between the two cultivars of *B. oleracea*, i.e., var. *italica* and var. *botrytis*, were significant. Differences in shoot regeneration were also observed in amphidiploids used as female parents. Natural *B. juncea* explants regenerated with the highest frequency followed by synthetic *B. napus*, whereas *B. carinata* showed the least regeneration response.

The interspecific crosses of *B. carinata* × *B. nigra* (BBC), *B. juncea* × *B. campestris* (AAB), and *B. napus* × *B. campestris* (AAC) showed superior regeneration response in that order (Table 1). The triploid BBC regenerated with the highest frequency of 65.9%, significantly exceeding the regeneration responses of both mid- and better parents. Similarly triploid AAB regenerated with a frequency significantly exceeding the mid- and better parents. Though the synthetic *B. napus* (AACC) regenerated with a frequency significantly inferior to its better regenerating parent *B. oleracea* (CC), the AAC triploid obtained from crossing synthetic *B. napus* with *B. campestris* regenerated with a frequency significantly superior to its mid-parental value. The regeneration response of the triploid combination ABC obtained either from the cross synthetic *B. napus* (AACC) × *B. nigra* (BB) or from the cross *B. juncea* (AABB) × *B. oleracea* (CC) was significantly lower than the mid- or better parent. Such a result might have arisen from negative interactions manifested in ABC triploids. But the regeneration response of ABBC tetraploid obtained from the cross *B. juncea* (AABB) × *B. carinata* (BBCC) was not only significantly superior to that of the two ABC triploid combinations but also that of their mid-parent. This suggests the absence of such negative interactions associated with the ABC triploid. The superior regeneration response of this ABBC tetraploid is possibly a consequence of the additional dose of B genome, which needs further investigation.

Shoot regeneration potential in various tissue explants of different *Brassica* species and cultivars have been examined (Dunwell 1981; Dietert *et al.* 1982; Murata and Orton 1987). However, the differences reported were not related to the experimental product of known diploids as is done in this study. Based on the interspecific cross combinations studied, the sesquidiploid BBC was found to have the highest regeneration response. This result indicates the possibility of improving regeneration response through reinforcing favourable parental genotypes and gene interactions.

Bingham, E. F., Hurley, I. V., Kaatz, D. M., and Saunders, J. W. 1975. Breeding alfalfa which regenerates from callus tissue in culture. Crop Sci. **15**: 719–721.

Bogani, P., Buiatti, M., Tegli, S., Pellegrini, M. G., Bettini, P., and Scala, A. 1985. Interspecific differences in differentiation and dedifferentiation patterns in the genus *Nicotiana*. Plant Syst. Evol. **151**: 19–29.

Buiatti, M., Baroncelli, S., Bennici, A., Pagliai, M., and Tesi, R. 1974. Genetics of growth and differentiation *in vitro* of *Brassica oleracea* var. *botrytis* II. An *in vitro* and *in vivo* analysis of a diallel cross. Z. Pflanzenzuecht. **72**: 269–274.

Caligari, P. D. S., Powell, W., and Goodall, V. 1987. The *in vitro* genetics of barley (*Hordeum vulgare* L.). Genetic analysis of immature embryo response to 2,4-dichlorophenoxy acetic acid. Heredity, **59**: 285–292.

Dietert, M. F., Barron, S. A., and Yoder, O. C. 1982. Effects of genotypes of *in vitro* culture in the genus *Brassica*. Plant Sci. Lett. **26**: 233–240.

Dunwell, J. M. 1981. *In vitro* regeneration from leaf disc of three *Brassica* species. J. Exp. Bot. **32**: 789–799.

Fazekas, G. A., Sedmach, P. A., and Palmer, M. V. 1986. Genetic and environmental effect on *in vitro* shoot regeneration from cotyledon explants of *Brassica juncea*. Plant Cell Tissue Org. Cult. **6**: 177–180.

Frankenberger, E. A., Hasegawa, P. M., and Tigchelaar, E. C. 1981. Diallel analysis of shoot forming capacity among selected tomato genotypes. Z. Pflanzenphysiol. **102**: 233–242.

Gomez, K. A., and Gomez, A. A. 1976. Statistical procedures for agricultural workers. John Wiley & Sons, New York.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**: 473–497.

Murata, M., and Orton, T. J. 1987. Callus initiation and regeneration capacities in *Brassica* species. Plant Cell Tissue Org. Cult. **11**: 111–123.

Narasimhulu, S. B., Prakash, S., and Chopra, V. L. 1988. Comparative shoot regeneration response of diploid *Brassica* and their synthetic amphidiploid products. Plant Cell Rep. **7**: 525–527.