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MICROPROPAGATION OF EARLY CABBAGES (*Brassica oleracea* var. *capitata*)

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Abstract

Seven genotypes of early cabbages (*Brassica oleracea* var. *capitata*), that represent prospective material for further breeding, were tested for their ability to regenerate shoots *in vitro*. Lateral buds from plants grown in the open field were used as explants. They were incubated on Murashige and Skoog's (MS) media supplemented with 1.0 and 2.0 mg l^{-1} of benzyladenine (BA) or 6-furfurylaminopurine (KIN) in combination with 0, 0.2, 0.5 and 1.0 mg l^{-1} indole-3-butyric acid (IBA). The BA-supplemented media were optimal for both growth and multiplication of shoots. The R_4 genotype had the highest multiplication index (MI) 8.96 on medium supplemented with 1.0 mgl^{-1} BA and 0.2 mgl^{-1} IBA, while the genotype R_{11} had the lowest MI 1.07 on medium with 2.0 mgl^{-1} KIN and 1.0 mgl^{-1} IBA. Rooting was performed in a media with different concentration of sucrose (2 and 4%) in combination with 0, 0.5 and 1.0 mgl^{-1} IBA. Shoots rooted maximally (100%) on all media and there was no statistical important influence of medium composition on rooting. Rooting plants were successfully acclimated and grown in greenhouse.

Key worlds: micropropagation, lateral buds, multiplication index, rooting

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is economically one of the most important varieties of *Brassica* genus. Cabbage contains the high amounts of vitamins C, K, A and folic acid, fiber, flavonoids, proteins and minerals. Because of good nutritional value and antioxidant and anti-inflammatory properties they are consumed worldwide in human diet. The influence of cabbage consumption on human health is evident and is, in addition to being a source of vitamins and fibre, connected with secondary metabolites called glucosinolates, which are known to possess anticarcinogenic properties (summarised by Sarikamis et al., 2009). Singh et al. (2006) found variability in antioxidant phytochemicals (ascorbic acid, lutein, β-carotene, DL-α- tocopherol and phenolics) in 18 cabbage cultivars.

Some improvements in agronomic and nutritional performances of existing genotypes of cabbage have been achieved through the years of conventional breeding. However, the improvement of these vegetables by conventional hybridization is complicated because of their two year head-seed-head cycle, problem with sporophytic incompatibility, requirement for isolation barriers etc.

Nowadays, many breeders attempt to improve *Brassica* crops by employing the biotechnological and genetic transformation approaches, in addition to the classic ones (reviewed by Vinterhalter et al., 2007). The successful application of these approaches requires efficient and reliable tissue culture regeneration system.

Shoot regeneration was achieved from various tissues and organs including hypocotyls, cotyledons, roots, leaves, peduncle segments, callus and cell cultures, thin cell

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layers and protoplasts (reviewed by Cardoza and Stewart, 2004). Regeneration in *B. oleracea* has been reported from leaf and root segments (Lazzeri and Dunwell, 1986), hypocotyls (Lillo and Shanin, 1986), cotyledons (Dale and Ball, 1991). However, considerable variation has been observed by different groups, even when working with the same species or variety.

As a part of a long-term project on improvements of *B. oleracea* varieties at Institute for Vegetable Crops in Smederevska Palanka, we found it necessary to investigate the shoot regeneration ability in *Brassica oleracea* var. *capitata* that represent prospective material for further breeding. We studied regeneration ability in seven genotypes of early cabbages.

Materials and methods

Lateral buds were excised from plants grown in the open field. Lateral buds were rinsed in 70% (v/v) ethanol for 1 min, surface sterilized in 20% commercial bleach (8% NaOCl) for 20 min, and then rinsed five times with sterile distilled water. The surface-sterilized buds were planted in Erlenmeyer flasks containing 50 ml of MS (Murashige and Skoog, 1962) medium containing 2% (w/v) sucrose, and 0.8% (w/v) agar (Torlak, Serbia) and supplemented with 1 mg l^{-1} BA and 0.2 mg l^{-1} IBA.

After that multiplication of induced shoots was analyzed on MS solid medium supplemented with 1 mgl^{-1} and 2 mgl^{-1} 6-benzyladenine (BA, Sigma Co., USA) or 6-furfurylaminopurine (kinetin, KIN, Sigma Co., USA) in combination with 0.2, 0.5 and 1.0 mgl^{-1} indole-3-butyric acid (IBA, Sigma Co., USA). MS medium without plant growth regulators (PGR) was used as control. Media pH was adjusted to 5.8 prior to autoclaving at 121 °C for 20 min. The cultures were maintained in a growth room under cool white fluorescent tubes and a 16 h day length, at 23 ± 2 °C.

Multiplied shoots reached 3 cm or more in height were cultured for four weeks on MS medium containing 2 or 4% sucrose and supplemented with 0.0, 0.5 and 1.0 mg l^{-1} IBA for rooting. Rooted shoots with three to five leaves were transplanted to pots containing soil for acclimation and cultured in growth chamber with high relative humidity (80%) for 3-4 weeks before moving to the greenhouse for further growth.

Results and discussion

The results shown that the BA-supplemented media were optimal for both growth and multiplication of shoots in all investigated genotypes (Table 1). The highest multiplication rate was ranged from 4.37 in R_1 to 8.96 in R_4 genotype. The genotype R_{11} had the lowest MI 1.07 on medium with 2.0 mgl^{-1} KIN and 1.0 mgl^{-1} IBA. Also the lowest index of multiplication was observed in all genotypes on media supplemented with KIN (without or in combination with IBA) and it was ranged from 1.07 in R_{11} to 5.06 in R_4 genotype. Obviously, R4 genotype had the highest IM on both media with BA (8.96) and KIN (5.06), while R_1 genotype had the weakest responses, 4.37 in media with BA and 1.18 on media with KIN.

The positive effects of BA on regeneration from wide range of explants were reported in different *Brassica* species. High frequency of regenerated shoots (100%) from hypocotyls using BA and NAA combination was achieved in *B. carinata* (Yang et al., 1991). Without addition of auxin NAA, 4.44 µM BA was the optimum concentration for shoot regeneration in *B. juncea* var. *tsatsai* (Guo et al. 2005). The presence of BA in the medium markedly increased the number of shoot produced per explant in rapid-cycling *B. oleracea in vitro* (Cheng et al., 2001).

On the other hand, we observed that BA-containing media caused hyperhydricity of shoots. On media with BA percentages of vitrification were from 9.6 to 75.11%. Especially high percents of shoot vitrification ware observed in four genotypes R4, R13, R16, and R22 (over 50%). Substitution of BA with KIN in media, reduced this percentage of vitrification under 50 % in all genotypes, but this substitution didn't give satisfactory multiplication results (IM from 1.07 to 5.06). Shoots vitrification on medium without PGR was observed in low percentage, 8 and 12.5% in R13 and R14 genotypes, respectively. Li et al. (2003) proposed that excess of cytokinins along with the high water potential of the medium were the major reasons for the vitrification of shoots.

Rooting was performed in a media with different concentration of sucrose (2 and 4%) in combination with 0, 0.5 and 1.0 mgl^{-1} IBA. Shoots rooted maximally (100%) on all media and there was no influence of medium composition on rooting (Table 2). Rooting plants were successfully acclimated and grown in greenhouse (Figure 1).

Conclusion

In conclusion, our results show a satisfactory frequency of shoot regeneration from lateral buds and multiplication of shoots on media containing 1 mgl^{-1} BA alone or in combination with IBA in seven investigated *B. oleracea* var *capitata* genotypes. Efficient *in vitro* plant regeneration, rooting, and acclimation protocol may be useful in breeding process developing new lines and cultivars in a shorter time and in genetic improvement by using biotechnological approaches.

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References

Cardoza, V., and Stewart J. R. N. (2004). Brassica biotechnology: Progress in cellular and molecular biology. In Vitro Cell. Dev. Biol.-Plant **40**, 542-551.

Cheng, P-K., Lakshmanan, P., and Swarup S. (2001). High-frequency direct shoot regeneration and continuous production of rapid-cucling Brassica oleracea in vitro. In Vitro Cell. Dev. Biol.-Plant **37**, 592-598.

Dale, P. J., and Ball L.F. (1991). Plant regeneration from cotyledonary explants in a range of
Brassica species and genotypes. Proceedings of Eight International Rapeseed Congress **4**, 1122-1127.

Guo, D-P., Zhu, Z-J., Hu, X-X., and Zheng, S-J. (2005). Effect of cytokinins on shoot regeneration from cotyledon and leaf segment of stem mustard (*Brassica juncea var. tsatsai*). Plant Cell Tissue Organ Cult. **83**, 123-127.

Lazzeri, P.A., and Dunwell, J.M. (1986). In vitro regeneration from seedling organs of Brassica oleracea var. italica Plenck cv Green Comet: I. Effects of plant growth regulators. Ann Bot **58**, 689-697.

Li, S., Li, W., Yang, D. L., and Cao, Z. Y. (2003). Advance of research vitrification in plant test-tube plantlets. J. Gansu Agric. Univ. **38**, 1-16.

Lillo, C., and Shanin, E. A. (1986). Rapid regeneration of plants from hypocotyl protoplasts and root segments of cabbage. Hort Sci. **21**, 315-317.

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

Sarikamiş, G.; Balkaya, A. and Yanmaz, R. (2009). Glucosinolates within a collection of white head cabbages (*Brassica oleracea var. capitata sub. var. alb*a) from Turkey. Afr J Biotechnol. 8, 5046-5052.

Singh, J.; Upadhyay, A.K.; Bahadur, A.; Singh, B.; Singh, K.P. and Rai, M. (2006). Antioxidant phytochemicals in cabbage (*Brassica oleracea L. var. capitata*). Sci Hort. 108, 233-237.

Vinterhalter, D., Sretenović-Rajičić, T., Vinterhalter, B., and Ninković, S. (2007). Genetic transformation of *Brassica oleracea* vegetables. Transgenic Plant J. **1**, 340-355.

Yang, M-Z., Jia, S.R., and Pua, E.C. (1991). High frequency regeneration from hypocotyls explants of Brassica carinata A. Br. Plant Cell Tissue Organ Cult. **24**, 79-82.

Table 1. Index of multiplication (IM) and percent of vitrification in MS media supplemented with different concentration of PGRs

MS medium	genotype	IM	vitrification (%)
PGR- free	R1	1.0	-
	R4	1.0	-
	R11	1.0	-
	R13	1.04	8.0
	R14	1.0	12.5
	R16	1.0	-
	R22	1.27	-
1.0 mgl^{-1} BA + 0.2 mgl^{-1} IBA	R1	4.31	22.62
	R4	8.96	75.11
	R11	6.88	38.37
	R13	6.7	57.66
	R14	4.41	30.00
	R16	7.06	57.94
	R22	5.45	38.22
1.0 mgl^{-1} BA + 0.5 mgl^{-1} IBA	R1	4.37	16.1
	R4	8.05	38.46
	R11	6.18	37
	R13	5.66	28.11
	R14	5.0	12.1
	R16	6.48	26.43
	R22	6.14	16.3
2.0 mgl^{-1} BA + 1.0 mgl^{-1} IBA	R1	3.37	44.4
	R4	8.6	31.4
	R11	4.6	37.5
	R13	6.56	33.3
	R14	5.7	9.6
	R16	8.54	50.52
	R22	7.8	55.08
1.0 mgl^{-1} KIN + 0.2 mgl^{-1} IBA	R1	1.2	16.67
	R4	1.84	22.86
	R11	1.21	2.6
	R13	1.44	38.46
	R14	1.17	23.8
	R16	2.61	41.96
	R22	1.95	-
2.0 mgl^{-1} KIN + 1.0 mgl^{-1} IBA	R1	1.18	23.08
	R4	5.06	31.34
	R11	1.07	12.5
	R13	1.81	36.73
	R14	1.39	16
	R16	2.47	26.58
	R22	1.35	18.52

Table 2. Frequency of rooting, number of roots per rooted shoot and average root lengths of regenerated shoots

Genotype	Sucrose % + IBA mg l^{-1}		Rooting, %	No. of roots ± SE*	Length of root ± SE, mm
R4	2%	0	100	5.33 ± 2.31	3.87 ± 0.31
		0.5	100	9.33 ± 2.31	3.53 ± 0.23
		1	100	10 ± 2	3.8 ± 0.87
	4%	0	100	9.0 ± 1.0	2.37 ± 1.25
		0.5	100	8.33 ± 2.52	3.13 ± 0.81
		1	100	8.67 ± 1.53	3.33 ± 0.81
R11	2%	0	100	10.0 ± 4.0	3.37 ± 1.77
		0.5	100	4.33 ± 2.52	3.07 ± 1.03
		1	100	6.67 ± 2.31	2.50 ± 1.61
	4%	0	100	14.0 ± 4.0	5.0 ± 0.53
		0.5	100	6.67 ± 3.51	3.47 ± 1.1
		1	100	12.0 ± 2.0	4.8 ± 2.6
R14	2%	0	100	5.67 ± 2.89	4.07 ± 1.45
		0.5	100	9.67 ± 2.52	3.0 ± 0.44
		1	100	8.0 ± 2.0	2.07 ± 0.31
	4%	0	100	7.67 ± 1.53	4.33 ± 0.83
		0.5	100	7.0 ± 2.65	3.63 ± 1.5
		1	100	7.0 ± 1.73	2.0 ± 0.17
R16	2%	0	100	8.0 ± 0.0	6.37 ± 1.19
		0.5	100	9.0 ± 1.73	6.0 ± 2.42
		1	100	9.33 ± 1.15	7.4 ± 1.56
	4%	0	100	7.33 ± 2.31	6.07 ± 1.72
		0.5	100	5.67 ± 2.89	7.3 ± 0.9
		1	100	8.67 ± 3.06	6.17 ± 0.06
R22	2%	0	100	12.67 ± 4.16	2.87 ± 0.55
		0.5	100	6.67 ± 1.05	3.73 ± 0.76
		1	100	9.0 ± 1.0	4.17 ± 0.25
	4%	0	100	5.33 ± 2.08	3.77 ± 1.02
		0.5	100	7.33 ± 1.15	4.9 ± 0.62
		1	100	8.67 ± 1.53	3.33 ± 0.81

*Value represents the mean ± standard error.

Figure 1. Successfully acclimated plants grown in greenhouse