UDC 575:635
DOI: 10.2298/GENSR1203611P
Original scientific paper

IN VITRO CULTURE AS A PART OF *BRASSICA OLERACEA* VAR. *CAPITATA* L. BREEDING

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Pavlovic S., S. Adžić, D.Cvikić, J. Zdravković and M. Zdravković (2012): *In vitro culture as a part of Brassica oleracea var. capitata L. breeding*. - Genetika, Vol 44, No. 3, 611 -618.

Fourteen genotypes of cabbage (*Brassica oleracea* var. *capitata* L.), that are a part of Institute for Vegetable Crops collection, were tested for their ability to regenerate shoots *in vitro.* Five of them are early, while nine are late genotypes. Lateral buds from plants grown in the open field were used as explants. In all genotypes, lateral buds showed the high percentage of shoot formation, ranged from 80% to 100%. They were incubated on Murashige and Skoog's (MS) media supplemented with 1.0 and 2.0 mgl^{-1} of benzyladenine (BA) or 1.0 mgl^{-1} 6-furfurylaminopurine (KIN) in combination with 0, 0.5 and 1.0 mgl^{-1} indole-3-butyric acid (IBA). The BA-supplemented media were optimal for both growth and multiplication of shoots. In both groups of genotypes, the highest index of multiplication (IM) was achieved on medium supplemented with 2.0 mgl^{-1} BA and 1.0 mgl^{-1} IBA, in R9 early genotype (IM 8.53) and K1 late genotype (IM

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10.06). R5 early and in K29 and K75 late genotypes had no multiplication on medium with 1.0 mgl^{-1} KIN (IM 1.00). Also, in all genotypes the lowest index of multiplication was observed on media supplemented with KIN (without or in combination with IBA).

Key words: cabbage, micropropagation, lateral buds, multiplication index

INTRODUCTION

Several Brassica species are widely grown as vegetable crops around the world. The cole crops (*Brassica oleracea* L.) and predominantly head cabbage (*Brassica oleracea* var. *capitata* L.) are among the world's most commonly cultivated vegetables (www.fao.org). Cabbage 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 (LORENZ and MAYNARD, 1988; RUBATZKY and YAMAGUCHI, 1997). The influence of cabbage consumption on human health is evident and is, in addition to being a source of vitamins and fiber, connected with secondary metabolites called glucosinolates, which are known to possess anticarcinogenic properties (summarized by SARIKAMIS *et al.,* 2009). There are clear indications that glucosinolates block tumor initiation and suppress tumors by apoptosis (MITHEN *et al.,* 2000). 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 breeding 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.

Plant regeneration systems for commercial micropropagation and disease-free plants production have been developed for a lot of *Brassica* vegetables. Shoot regeneration was achieved from various tissues and organs. Plant regeneration from cultured tissues through adventitious bud formation has been developed in various *B. oleracea* varieties, including cabbage (RADCHUK *et al.,* 2000; MUNSHI *et al.,* 2007; PAVLOVIC *et al.,* 2010).

Regeneration in *B. oleracea* has been reported from leaf and root segments (LAZZERI and DUNWELL, 1986; CAO and EARLE, 2003), hypocotyls (LILLO and SHANIN, 1986; METZ *et al*., 1995; PUDDEPHAT *et al.,* 2001, HAZRAT *et al.,* 2007), cotyledons (DALE and BALL, 1991, HAZRAT *et al,* 2007), peduncle explants (CHRISTEY and EARLE, 1991). However, considerable variation has been observed by different groups, even when working with the same species or variety.

Regeneration of whole plants from cultured tissues or cells is a prerequisite for successful applications of *in vitro* techniques of gene transfer, mass propagation and somaclonal variation studies.

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* L. that represent prospective material for further breeding. We studied regeneration ability in fourteen genotypes of cabbages.

MATERIALS AND METHODS

Lateral buds were excised from plants grown in the open field. In order to product lateral buds-donor plants, cabbage seeds have been sown in plastic greenhouse in middle of Jun 2009. After about one month obtained nursery plants were transplanted into open field where cabbage heads have been formed. Complete cabbage heads were formed in second half of September and in first half in October for early genotypes and in second half of October for late genotypes. Genotypes were selected according to morphological characteristics and included as prospective material in further breeding.

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. 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.

Multiplication of induced shoots was analyzed on MS solid medium supplemented with 1.0 mgl^{-1} and 2.0 mgl^{-1} 6-benzyladenine (BA, Sigma Co., USA) or 1.0 mgl^{-1} 6-furfurylaminopurine (kinetin, KIN, Sigma Co., USA) in combination with 0.0, 0.5 and 1.0 mgl^{-1} indole-3-butyric acid (IBA, Sigma Co., USA). MS medium without plant hormones was used as control. Index of multiplication (IM) was calculated as the mean number of shoots per explant after four weeks of culture on multiplication media.

The data were subjected to analysis of variance (factorial ANOVA). Percentage data were transformed via arcsine before analysis. The means were separated using Fisher's LSD test at $P < 0.05$.

RESULTS AND DISCUSSION

The cytokinins were used for *in vitro* shoot regeneration and five treatments were tested. Without the application of cytokinins, on hormone free medium, shoots were formed in 2 from fourteen genotypes with low index of multiplication, 1.17 and 1.5 in R33 and K50 genotypes, respectively.

ANOVA showed that both factors (genotype and media) and their interactions, significantly affected the mean number of shoots per explant. There

were no statistical significant differences between groups of early and late genotypes.

In all genotypes, lateral buds showed the high percentage of shoot formation, ranged from 80% to 100%. Genotypes have been more productive in terms of shoots multiplication on media supplemented with BA than on media supplemented with KIN, but different genotypes responded with different intensity. Index of multiplication was ranged from 2.0 to 10.06 on media with BA, and from 1.07 to 2.5 on media with KIN (Table 1).

Table 1. Index of multiplication (IM) and percentage of shoots vitrification in MS media supplemented with different concentration of plant hormones

MS medium	1.0 mgl^{-1} BA		1.0 mgl^{-1} BA + 0.5 mgl^{-1} IBA		2.0 mgl^{-1} BA + 1.0 mgl^{-1} IBA	
Genotype	IM[a]	% vitrification	IM	% vitrification	IM	% vitrification
R1	3.15 op	15.87	2.60 lm	11.54	3.88 rs	29.03
R5	2.00 i	20.00	3.27 p	36.73	4.33 v	18.46
R7	2.75 mn	9.09	6.33 A	27.37	4.80 w	27.78
R9	4.86 w	35.51	4.13 tu	22.58	8.53 D	69.53
R33	4.60 w	63.04	6.13 z	38.04	6.73 C	75.25
K1	3.62 q	55.26	6.80 C	58.82	10.06 E	81.77
K6	2.81 n	26.67	4.20 uv	53.97	3.87 rs	65.52
K7	2.50 lm	12.50	2.36 jk	30.30	3.13 op	62.00
K23	6.65 BC	17.08	6.80 C	52.94	5.87 y	63.64
K29	3.75 uv	46.67	3.67 q	47.27	3.70 q	72.97
K35	6.60 lm	61.36	4.21 u	20.34	5.27 x	79.75
K48	4.25 o	63.53	4.00 st	53.33	3.19 op	45.10
K50	2.63	18.00	2.00 i	31.30	4.80 w	48.61
K75	3.09	28.17	4.00 s	47.50	2.67 lmn	31.25

MS medium	1.0 mgl^{-1} KIN		1.0 mgl^{-1} KIN + 0.5 mgl^{-1} IBA	
Genotype	IM	% vitrification	IM	% vitrification
R1	1.14 ab	-	1.07 ab	-
R5	1.00 a	-	1.33 cd	-
R7	1.50 ab	-	1.13 fg	-
R9	2.50 kl	32.00	1.4 g	4.76
R33	1.50 ab	-	1.13 fg	-
K1	2.00 i	20.00	1.87 hi	21.43
K6	2.20 j	31.25	1.12 ab	-
K7	1.07 ab	-	1.07 ab	-
K23	1.70 g	11.76	1.27 bc	-
K29	1.00 a	-	1.07 ab	-
K35	1.4 cdf	-	1.07 ab	-
K48	1.70 h	-	1.4 g	-
K50	1.13 ab	-	1.4 cdf	14.29
K75	1.00 a	-	1.33 df	-

[a] Numbers followed by a different letter are significantly different at $P < 0.05$ according to the LSD test.

The highest multiplication rate was ranged from 3.13 in K7 genotype to 10.06 in K1 genotype, both on media supplemented with 2.0 mgl^{-1} BA + 1.0 mgl^{-1} IBA. K1 genotype had also the best response on two more media supplemented with 1.0 mgl^{-1} BA + 0.5 mgl^{-1} IBA, and 1.0 mgl^{-1} KIN + 0.5 mgl^{-1} IBA. In all genotypes the lowest index of multiplication was observed on media supplemented with KIN (without or in combination with IBA) and it was ranged from 1.07 in to 2.5. The lowest IM (1.07) was observed in five genotypes, in four on media with 1.0 mgl^{-1} KIN and 0.5 mgl^{-1} IBA and in one genotype on medium with 1.0 mgl^{-1} KIN. The genotype with the lowest response on all media was K7 genotype, which index of multiplication was ranged from 1.07 to 3.13. In tree genotypes on medium with 1.0 mgl^{-1} KIN no multiplication was observed.

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 vitrification of shoots. On media with BA percentages of vitrification were from 9.09% in R7 genotype on medium with 1.0 mgl^{-1} BA to 81.77% in K1 genotype on medium with 2.0 mgl^{-1} BA + 1.0 mgl^{-1} IBA (Table 1). Especially high percentages of shoot vitrification were observed in nine of fourteen genotypes (over 50%). Substitution of BA with KIN in media, reduced this percentage of vitrification under 50 % in all genotypes (from 4.76% to 32%), but this substitution didn't give satisfactory multiplication results (IM was ranged from 1.07 to 2.5). Shoots vitrification on medium without PGR was no observed. 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.

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 fourteen investigated *B. oleracea* var *capitata* L. genotypes. Efficient *in vitro* plant regeneration protocol may be useful in breeding process developing new lines and cultivars in a shorter time and in genetic improvement by using biotechnological approaches.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education and Science of Republic of Serbia Grants No. TR31059.

Received September 30th, 2011
Accepted September 07th, 2012

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IN VITRO KULTURA KAO DEO PROCESA OPLEMENJIVANJA *BRASSICA OLERACEA* VAR. *CAPITATA* L.

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Kod četrnaest genotipova kupusa (*Brassica oleracea* var. *capitata* L.), koji su deo kolekcije Instituta za povrtarstvo, ispitana je sposobnost *in vitro* regeneracije pupoljaka. Ranih kupusa je bilo pet gonotipova, dok je kasnih kupusa bilo devet genotipova. Bočni pupoljci biljaka gajenih na otvorenom polju korišćeni su kao eksplantati. Kod svih genotipova bočni pupoljci su pokazali visok procenat regeneracije pupoljaka, od 80% do 100%. Inkubirani su na Murashige and Skoog's (MS) hranljivoj podlozi sa dodatkom 1.0 i 2.0 mgl^{-1} benziladenina (BA) ili 1.0 mgl^{-1} 6-furfurilaminopurina (KIN) u kombinacija 0, 0.5 i 1.0 mgl^{-1} indole-3-butirične kiseline (IBA). Podloge koje su sadržale BA su bile optimalne, kako za regeneraciju pupoljaka, tako i za njhovu kasniju multiplikaciju. Kod obe grupe genotipova najveći indeks multiplikacije je postignut na podlozi sa dodatkom 2.0 mgl^{-1} BA i 1.0 mgl^{-1} IBA, kod R9 ranog genotipa (IM 8.53) i kod K1 kasnog genotipa (IM 10.06). Kod R5 ranog genotipa i kod K29 i K75 kasnih genotipova nije bilo multiplikacije na podlozi sa 1.0 mgl^{-1} KIN (IM 1.00). Takođe najmanji indeks multiplikacije kod svih genotipova je primećen na podlogama sa KIN (bez ili u kombinaciji sa IBA).

Primljeno 30. IX. 2011.
Odobreno 07. IX. 2012.