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Full Length Article

Effects of Colchicine and High Temperature Treatments on Isolated Microspore Culture in Various Cabbage (*Brassica oleraceae*) Types

BURCU TUNCER[1] AND RUHSAR YANMAZ†
Yüzüncü Yıl University, Agricultural Faculty, Department of Horticulture, 65080, Van, Turkey
†Ankara University, Agricultural Faculty, Department of Horticulture, 06110, Ankara, Turkey
[1]Corresponding author's e-mail: brctuncer@gmail.com

ABSTRACT

The effect of colchicine with heat shock treatments on the microspore embryogenesis were evaluated in white head cabbage (*B. oleracea* var. *capitata* subvar. *alba*), kale (*B. oleracea* var. *acephala*) and ornamental kale (*B. oleracea* var. *acephala*). 2 different colchicine doses (50 & 100 mg/L) applied to the isolated microspores in order to stimulate the embryo induction for 15 h. After colchicine treatments microspores were exposed to 32°C and 35°C temperature in NLN-13 medium (40,000 microspore/mL) for 48 h. According to the 12 day and 19 day embryo yields after planting 50 mg/L colchicine treatment was found more effective. 32°C+50 mg/L colchicine treatment (5.3 embryo/petri) in white head cabbage (Yalova-1) and 35°C+50 mg/L colchicine treatment (9.4 embryo/petri) in ornamental kale were found effective treatments, respectively. White head cabbage (Erçiş genotype) and kale did not give positive respond to the colchicine treatments. © 2011 Friends Science Publishers

Key Words: *Brassica oleracea*; Microspore embryogenesis; Heat shock; Colchicine

INTRODUCTION

Anther and microspore cultures can be utilized in order to shorten the period of breeding studies in *Brassica* species for obtaining haploid embryos. It is noted that microspore culture studies on *Brassica* species can be successfully applied on rape (Zhang & Takahata, 2001; Segui-Simarro *et al.,* 2003; Weber *et al.,* 2005), mustard (Barro & Martin, 1999), Chinese cabbage (Sato *et al.,* 2005), broccoli, cauliflower, white head cabbage, savoy cabbage, brussels sprout (Takahata & Keller, 1991; Duijs *et al.,* 1992; Ferrie *et al.,* 1999; Dias & Correia, 2002) and ornamental kale (Zhang *et al.,* 2008) but success vary by the genotype, growing conditions of donor plant, microspore developmental stage, medium contents, culture conditions and external treatments.

High temperature treatment can be effectively used in the microspore embryogenesis induction studies on different *Brassica* types. More successful results are noted to be attained 30°C for 2 days in *B. napus* (Wan *et al.*, 2011), 30°C for 2 days in broccoli (Duijs *et al.,* 1992), 33ºC for 1 day in Chinese cabbage (Cao *et al.,* 1994), 32ºC for 2 days in cauliflower, white head cabbage and *B.oleracea var. sabauda* (Ferrie *et al.,* 1999), 32.5°C for 1 day in *B. oleracea var costata* (Dias & Correia, 2002).

Colchicine treatment in *Brassica* species is a stress factor stimulating embryo formation. Application of 50 and 500 mg/L colchicine doses to the microspores isolated in *B.napus* for 15 h affected the embryo formation and embryo quality positively (Zhou *et al.,* 2002). Zhang *et al.* (2008), applied 50 mg/L colchicine for 2 days to the microspores isolated from 29 different ornamental kale varieties. They concluded that embryo formation ratio were 19.0 to 43.4 embryo/petri according to the genotypes.

The aim of this study was to search effective colchicine with high temperature treatments for obtaining haploid embryos by microspore culture with Turkish head cabbage and kale varieties and one ornamental kale varieties.

MATERIALS AND METHODS

Plant materials: Three species were selected including white head cabbage (Erciş genotype & cv. Yalova-1), kale (inbred line) and ornamental kale cv. Chidori Red F1.

Donor plants and growth conditions: White head cabbage and kale seeds were sown on first week of May 2008 and ornamental kale seeds were sown in August 2008 on peat-filled viols. Kale and white head cabbage seedlings were planted when 3-4 leaf stages with intervals of 60-80 cm within June 2008, and ornamental kale seedlings in September 2008 in the field at intervals of 30-60 cm. Cabbage heads and kale plants were harvested end of November 2008 and were stored in an unheated greenhouse

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in trenches to the spring while ornamental kale plants were left in the field from winter to spring and then buds were collected after the flowering.

Microspore isolation: Flower buds containing microspores at the late uninucleate stage of development were used. Buds were sterilized in 10% sodium hypochlorite solution for 10 min., then rinsed with sterile, bidistillated water 3 times for 6 min. Forty flower buds were used in each isolation. The buds were macerated with a glass rod in 3.5 mL NLN medium (Lichter 1982) containing 13% sucrose (hormone-free, pH 6.1) and microspores were made free. After that, microspore suspension was sifted through sieves with 40 µm holes, the residue on the sieve and beaker were washed again with 6.5 ml NLN-13 medium and transferred to glass beakers. Sieved suspension was taken in centrifuge tubes, centrifuged in 900 rpm speed in 4°C 3 times for 3 min each and microspore residue was attained.

Colchicine treatment: A 0.2% stock solution of colchicine in the induction medium (NLN-13) was prepared and filtersterilized. Appropriate volumes of stock solution were added to the culture medium (NLN-13) immediately after microspore isolation in order to give the various desired colchicine concentrations (50 & 100 mg/L). Later, 1 mL colchicine solution per bud was added to the pellet obtained after the last centrifuge process as explained above and it was suspended. After colchicine treatment in darkness at 30°C for 15 h, the microspores were washed twice by centrifuging the microspore suspension with fresh induction medium.

Temperature treatment and culture of microspores: Microspores were resuspended in fresh NLN-13 medium (40,000 microspore/mL). Five-mililiter aliquots of microspore suspension were dispensed into 60 mm × 15 mm sterile petri dishes (200,000 microspore/petri). Dishes were incubated in the dark at 32°C and 35°C for 48 h to induce embryogenesis, and then at 25°C in the dark conditions. When 2 or more embryos were seen with naked eye (approx. 11-12 days after the isolation) petri dishes were taken on the orbital shaker (45 rpm) and kept for 3 weeks under dark conditions in 25°C.

Performing embryo yield: Embryo counts per petri dish were made 2 (12^{th} day) and 3 (19^{th} day) weeks after the isolation under the binocular microscope.

Statistical analysis: Embryos were counted per petri dish (8 petri dishes for each treatment, 4 repetitions for treatment and totaly 32 petri dishes per treatment). The obtained data were analyzed using factorial variance analysis (temperature x colchicine treatments). Differences among temperature and colchicine treatments were tested using Duncan's multiple range test at p=0.05. Statistical analysis was performed using SPSS statistical package program.

RESULTS AND DISCUSSION

Results showed that colchicine doses (50 & 100 mg/L) were not effective alone, its effects differed according to the temperature treatments. Temperature colchicine interaction was found statistically significant ($p<0.05$). Embryo yield increased in 32°C + 50 mg/L colchicine treatment (6.59 embryo/petri) in Yalova-1 white head cabbage to the control group (5.23 embryo/petri) and 100 mg/L colchicine treatment in Erciş genotype (4.19 embryo/petri). However, 100 mg/L colchicine treatment was found effective in both species at 35°C (Table I).

According to the embryo yields performed 19^{th} day, it draws attention that results are similar to the results of 12^{th} day. The highest embryo still attained from 32°C + 50 mg/L dose in Yalova-1 white head cabbage type (Table I).

According to results of 12^{th} day in kale, the highest embryo acquired from control in 35°C (6.03 embryo/petri). 32°C + 100 mg/L (4.99 embryo/petri) colchicine dose succeeded thereafter. 50 mg/L colchicine dose was not found effective (Table II).

As for the ornamental kale, whereas the difference among the temperatures in embryo yield was not found statistically significant; the difference among the colchicine doses was found significant ($p<0.05$). It is seen that control and 50 mg/L colchicine treatments in 32°C do take place in the same statistical group, though the difference between all doses in 35°C are significant (Table III). The highest embryo yield was obtained from 35°C + 50 mg/L colchicine treatment (11.59 embryo/petri) and 32°C + 50 mg/L (8.09 embryo/petri) treatment succeeded it. On the other hand, 100 mg/L colhicine dose had a preventive impact on embryo formation both temperatures (Table III). According to the embryo at the end of 19^{th} day, favorable results shared similarity with the results of 12th day. On the 19^{th} day of culture period, it draws attention that embryo formation values reduced in all doses apart from control dose (Table III).

Microscopic examinations revealed that approximately 3 weeks after the isolation (19^{th} day), heart (Fig. 1a), torphedo (Fig. 1b), walking-stick shaped embryos (Fig. 1c) and developed embryos (Fig. 1d) were observed in cultures.

Colchicine treatments have been focused on the *Graminaea* and *Brassicacea* families, in which microspore embryogenesis is already successful (Shariatpanahi *et al.*, 2006). In different species more successful results are noted to be obtained 50 mg/L colchicine for 48 h in ornamental kale (Zhang *et al.*, 2008), 100 mg/L colchicine for 48 h in coffee (Herrera *et al.*, 2002), 3 mM colchicine for 24-48 h in wheat (Hansen & Andersen, 1998). Earlier studies showed that colchicine treatments adapted embryo formation from microspores particularly in *B. napus* (Zhao & Simmonds, 1995; Zhou *et al.,* 2002; Weber *et al.,* 2005), and ornamental kale (Zhang *et al.,* 2008) being members of *Cruciferae* family. Colchicine treatment of isolated *B. napus* microspores with the concentrations 50 and 500 mg/L for 15 h stimulated embryogenesis and produced large amounts of healthy-looking embryos and high doubling efficiency of 83–91% (Zhou *et al.,* 2002).

Table I: Effect of colchicine and high temperature treatments in white head cabbage (Yalova-1 and Erciş genotype) on embryo yield (12th and 19th)

Accessions	Colchicine dose (mg/L)	32°C		35°C	
		Mean yield (embryos/petri[1)])		Mean yield (embryos/petri[1)])	
		12th day	19th day	12th day	19th day
White head cabbage					
	Control	2,66±1,00 b B [1]	3,80±1,42 a A [1]	2,53±0,59 a AB [1]	2,36±0,85 a A [1]
Yalova-1	50	6,59±1,48 a A [1]	4,06±0,76a A [1]	1,06±0,35 a B [2]	0,93± 0,28a A [2]
	100	2,16±0,36 a B [1]	1,91±0,71a A [1]	4,86±2,32 a A [1]	3,10±1,17 a A [1]
	Control	5,23±2,33 a A [1]	3,45±1,49a A [1]	0,36±0,28 a B [2]	0,43±0,24 a A [2]
Erciş Genotype	50	0,79±0,27 b B [1]	0,66±0,28b B [1]	1,56±0,53 a AB [1]	1,52±0,61 a A [1]
	100	4,19±0,71 a A [1]	2,91±0,65 a AB [1]	3,92±0,52 a A [1]	2,86±0,55 a A [1]

[1]5 mL NLN-13 / 60 x 15 mm petri dish (40.000 microspore/ml=200.000 microspore/petri dish)
Different capital letters in a same column show significant differences among the colchicine doses ($p<0.05$)
Different small letters in a same colchicine dose show significant differences among the species ($p<0.05$)
Different figures in a same line show significant differences among the temperatures ($p<0.05$)

Table II: Effect of colchicine and high temperature treatments in kale on embryo yield (12th and 19th)

Accessions	Colchicine dose (mg/L)	32°C		35°C	
		Mean yield (embryos/petri[1)])		Mean yield (embryos/petri[1)])	
		12th day	19th day	12th day	19th day
Kale					
	Control	3,12±0,75ab	0,96±0,54ab	6,03±1,78a	4,06±1,64a
Inbred line	50	0,36±0,14b	0,30±0,12b	0,93±0,85b	0,43±0,43b
	100	4,99±0,94a	3,42±1,37a	3,99±1,47ab	2,73±0,99ab

Table III: Effect of colchicine and high temperature treatments in ornamental kale on embryo yield (12th and 19th)

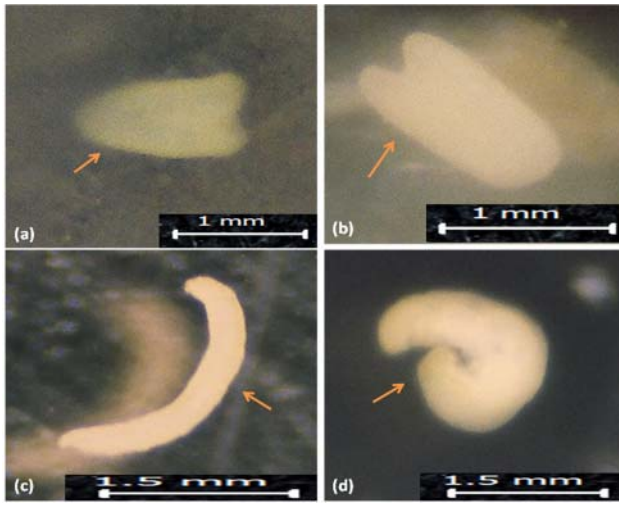
Accessions	Colchicine dose (mg/L)	32°C		35°C	
		Mean yield (embryos/petri[1)])		Mean yield (embryos/petri[1)])	
		12th day	19th day	12th day	19th day
Ornamental Kale					
	Control	7,46±2,16a	8,46±2,19a	7,13±0,69b	7,13±1,37a
Chidori Red F1	50	8,09±1,22a	5,23±1,18a	11,59±1,92a	7,16±1,06a
	100	0,22±0,10b	0,18±0,08b	0,21±0,12c	0,18±0,09b

[1]5 mL NLN-13 / 60 x 15 mm petri dish (40.000 microspore/ml=200.000 microspore/petri dish)
Different letters in a same column show significant differences among the colchicine doses ($p<0.05$)
There was no significant difference between the temperatures

In the researched said, mostly 50-500 mg/L colchicine doses were suggested. In our study, these source statements were taken into consideration and 50 and 100 mg/L doses were applied. The effect of the colchicine treatment varied with temperature. Accordingly, the most favorable results were achieved with 32°C + 50 mg/L colchicine treatment (6.59 embryo/petri) in Yalova-1 white head cabbage. Colchicine treatment was not found effective in Erciş genotype and kale (Table I & II). On the other hand, stimulatory effect of 50 mg/L colchicine dose was visibly seen on ornamental kale in both temperatures (Table III). Nonetheless, 100 mg/L dose was found to be unsuccessful. Results from ornamental kale were harmonious with data of Zhang *et al.* (2008). Results of others were not regarded as harmonious due to the difference in species since they were not carried out on *Brassica napus* species (Zhao & Simmonds, 1995; Zhou *et al.*, 2002; Weber *et al.*, 2005).

Performing the embryo yields at 2 different times is significant for determining the changes that occurred in the structures and quality of embryos in one week. While embryo yield was higher at the end of 12th day in all treatments, it was detected that the structure and quality of

Fig. 1: 19-day-old; (a) heart embryo (32°C + 50 mg/L colchicine, kale); (b) torphedo embryo (32°C + 50 mg/L colchicine, Yalova-1); (c) walked stick embryo (32°C + control , ornamental kale); (d) developed embryo (32°C + 50 mg/L colchicine, ornamental kale)



embryos was damaged due to the infection found in cultures in the counts on the 19th day and that there was a decrease compared to the 12th day.

CONCLUSION

Even though it was revealed that the effect may vary by temperature degree and colchicine dose types according to the findings of the research, studies are need to be made on many genotypes of a single species in future. As a result of this study, Erciş genotype (32°C+control) and kale (35°C+kontrol) colchicine doses were not effective on microspore embryogenesis. On the other hand, Yalova-1 head cabbage (32°C+50 mg/L), ornamental kale both degree of temperature 50 mg/L colchicine dose found to be effective in stimulating the formation of the embryo.

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