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RELATIONSHIP BETWEEN GENOTYPE AND LOW TEMPERATURE ON CALLUS FORMATION AND ROOT DEVELOPMENT THROUGH ANTHER CULTURE OF BROCCOLI "*Brassica oleraceae* L.var.italica "

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ABSTRACT: ***Anther culture was used for studying the effect of chilling treatment (4^{o}C for 24 hrs) of flower buds after sterilization on the callus differentiation in five hybrids of Broccoli Brassica oleraceae L.var.italica i.e., Sunrise and Dome from Holland, Landmark and Pinnacle from U.S.A., and Comanche from Japan.***

Flower buds were collected at the onset of flowering before elongation of the infloresence axis from plants grown in the end of Dec. 2006 to end Jan. 2007 in the open field at Dokki location and exposed to low temperature (4^{o} C)for 24 hrs after sterilization , in addition to the control treatment at room temperature (25^{o} C).

Miller medium (1963) supplemented with (2ppm) of each of 2,4-D and IAA , and (1ppm) kinetin with (1ppm) yeast extract was used for anther culture . Moreover, 2,4-D was excluded and IAA beside kinetin concentration were decreased to (0.5ppm) in the medium used for differentiation .

The obtained results showed a rise in anther response produced callus, anther response produced root and callus response developed root characters reached 45.5, 10.8 and 22.4% for low temperature respectively, while they were 31.7 , 5.6 and 15.3 % under room temperature conditions. The variability in the response of anther culture for the studied characters differed significantly according to genotypic differences. Sunrise hybrid gave the highest values for the three studied characters (59.2, 21.2 and 35.3%), respectively. While the lowest values were (21.03, 4.0 and 8.6 %) recorded in pinnacle, Comanche and Dome hybrids for anther produced callus, anther produced root and callus developed root characters, respectively.

The interaction effect showed that Sunrise gave the highest values of anther produced callus, anther produced root and callus developed root characters for low and room temperature, while, Dome showed a better response to cold pretreatment values for the three studied characters.

Key Words: ***Anther culture, Broccoli, buds chilling, genotype, callus induction, root development.***

INTRODUCTION

Anther culture technique has been applied extensively in some Brassica oleraceae cultivars viz. cabbage, Cauliflower, Brussels sprouts, Kohlrabi,

Chinese kale, Kales and Broccoli as reported by Arnison and Keller, (1990), Arnison *et al,* (1990), You-Ming *et al*., (1996), Vyvadilova *et al*, (1998,a) and Krzyzanowska, (2005) However, little information are available on the effect of low temperature on broccoli anther culture for callus formation and differentiation.

Callus induction and root differentiation from anther culture of broccoli were reported with Nishi *et al*. , (1974), Tzen and Lin, (1975), Quazi, (1978) Zhong and Cheng (1981) and You-Ming *et al.*, (1996). Meanwhile, differentiation of root was observed in callus from anther culture of cauliflower "Brassica oleraceae var. botrytis " with Bagga *et al*. ,(1982) .

Arnison *et al*., (1990), and Zhang *et al*., (1999), mentioned that, the rate of anther culture response was successfully affected by high temperature treatment in broccoli, but Hamaoka *et al,* (1991) indicated that hight temperature for the first 24 hrs. in anther culture of Brassica campestris subsp. Pekinensis inhibited normal pollen development and induced abnormal symmetrical division. They suggested that normal pollen differentiation is blocked by high temperature. Meanwhile, cold pretreatment was reported to have the advantage of more symmetric divisions Nitsch, (1974), delay of pollen or anther wall senescence Sunderland, (1978) and reducing the percentage of abnormal microspores Qu and Chen, (1983). Cold treatment before anther or microspore isolation in same Crusiferae crops including broccoli demonstrated by Modyaeva (1990), Osolnik *et al*., (1993), Achar, (2002), Nada *et al*., (2003) and Gorecka and Krzyzanowska, (2004) .

The aim of this work was focused on the effect of low temperature (4^{o} C for 24 hours) of flower buds after sterilization on callus differentiation in five hybrids of Broccoli. In addition, the relationship between genotype and temperature on anther culture was recorded.

MATERIALS and METHODS

1-Plant materials:-

Five F1 hybrid seeds of Broccoli were obtained from U.S.A. "Landmark and Pinnacle", Japan "Comanche "and Holland "Sunrise and Dome". The transplants F1 hybrids were planted in open field by 20th of Sept. 2oo6 at the experimental site of Dokki Veg. Res. Hort. Res. Inst. Giza Govn., in clay soil, four plants from each hybrid were selected for the development of superior quality head and kept as a donor for anther culture .

2-Chilling treatment:

Flower buds were selected (2.5-3mm) from the main inflorescences prior to flower emergence, and surface sterilized by 10% clorax solution (0.5 % sodium hypochlorite) with one drop of Tween 20/100 ml. for 15 min. followed by three washes in sterile distilled water, and it was exposed to a cold

treatment in a refrigerator for 24 hrs at 4°C before anther excision. Meanwhile in the case of room temperature treatment , the anthers were cultured after buds sterilization .

Under a laminar flow cabinet at Dokki tissue culture lab. Around 6-9 anthers were cultured without filaments in 200 ml. jars then sealed by with parafilm. Each jar contained 15 ml. of the culture medium as described by Miller, (1963) supplemental with 2ppm of each of 2,4-dichlorophenoxy acetic acid and indol-3-acetic acid plus 1ppm of 6-furfuryl amino purine, beside 1ppm yeast extract for initial culture of anther . The hormones were 0.5ppm of each of indol-3-acetic acid (IAA) and kinetin in the medium used for differentiation . The pH medium was adjusted at 5.7 and the media were sterilized by autoclaving at 121°C for 20 min.

The anthers were cultured in the absence of light at 25°C. The calli (fig.1) were transfered after 4 weeks from culturing the anthers to 250 ml. jars containing 50 ml. of IAA / Kinetin medium for differentiation . The calli were maintained in growth room at 25°C with 16 hours of cool white illumination of 31 ME m-2 (-1500 lux). Within 8 weeks some of this calli developed roots (fig.2, a&b) only and others did not differentiate .

Experimental Design :-

A completely randomized design in a factorial arrangement Cochran and Cox, (1957) was followed with two factors, i.e. hybrids and temperatures with four replicates for each treatment. The number of jars for each treatment were 40, 24,12,60 and 32 for pinnacle, Comanch, Dome, Landmark and Sunrise hybrids, respectively

The following data were recorded:-

1-Anther producing callus, (the frequency of anthers producing callus after four weeks from date of anther culture).

2- Anther producing roots, (the frequency of anther producing roots from callus differentiation within 8 weeks from the start of differentiation).

3-Callus to developing roots, (the frequency of calli developing roots within 8 weeks from the start of differentiation).

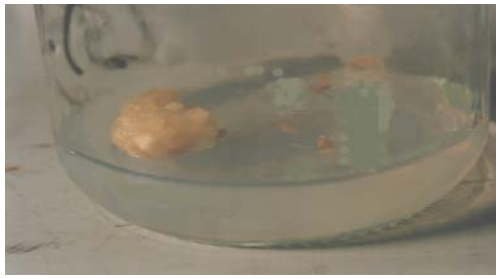
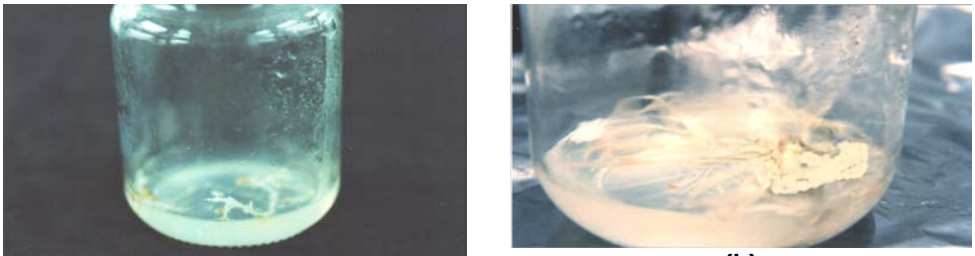


Fig. (1)callus formation from anther culture



(a) **(b)**

Fig. (2, a-b) Developing roots from callus differentiation

RESULTS AND DISCUSSION

Genotypic effect on callus formation, rooting percentage and root development of Broccoli:-

Significant genotypic differences on callus formation, rooting percentage and root development characters have been observed in Table (1) .Sunrise hybrid gave the highest response than the other tested hybrids for all studied characters. On the other hand, Pinnacle hybrid gave the lowest value of callus formation character.

As for rooting percentage, results in Table (1), show clearly that the highest values were detected to sunrise hybrid. On the other side, the lowest values were obtained from pinnacle and Comanche hybrids without significant differences between them. This character was increased by 79.5, 14.8 and 1.0% in Landmark, Dome and pinnacle compared with Comanche.

Regarding the effect of genotype on root development during rooting stage, results in Table (1) indicate that significant differences were found among the tested genotypes in this character, the highest values were obtained from Sunrise hybrid. On the other hand the lowest values were reported from Dome hybrid. In the same Table (1), the callus developing root character was increased by 111.5, 85.0 and 60.2% in the Pinnacle, Comanche and Landmark hybrids respectively compared with Dome hybrid.

Table (1): Genotypic effect on callus formation and root development of Broccoli.

F1 hybrid	Callus formation%	Rooting percentage	Root development%
Pinnacle	21.03	4.04	18.19
Comanche	24.58	4.00	15.91
Dome	36.19	4.59	8.60
Landmark	52.09	7.18	13.78
Sunrise	59.16	21.16	35.33
LSD(0.05)	0.60	0.20	0.87

From the above mentioned results in Table (1), its clear that, the variability in the tested characters of the anther culture might have a genetic basis and probably due to genetic constituents for each hybrid. Genotypic differences have been observed in anther culture of a number of Brassica spp. including broccoli as found by Arnison and Keller (1990), Fuller and Turton ,(1990). For example, You-Ming *et al.,* (1996), worked on 11 varsities anther culture of broccoli and found that the callus formation rates were ranged from 0 to 47.3% varied with verities.

Low temperature effect on the callus formation, rooting percentage and root development of Broccoli :-

The response of callus formation, rooting percentage and root development were increased significantly with a cold pretreatment period of 4 ^{o}C at 24 hrs on flower buds for the five tested hybrids, (Table 2). It reached 45.5%, 10.6% and 22.4% under low temperature condition. While it was 31.7%, 5.6% and 15.3% under room temperature respectively, it is means that, 48.3 percent of the calli differentiated roots in the absence of cold pretreatment. Similar results have been reported by Quazi, (1978) mentioned that fifty percent of the calli from anther culture of broccoli differentiated roots only.

Table (2): Effect of low temperature on callus formation and root development of anther culture in Broccli.

Treatments	Callus Formation %	Rooting Percentage %	Root Development %
Room (25 ^{o}C)	31.7	5.6	15.3
Low (4 ^{o}C)	45.5	10.6	22.4
LSD (0.05)	0. 4	0.1	0.6

The low temperature exhibited 43.4%, 90.6% and 46.7% over that of room temperature for the three tested characters respectively .The cold pretreatment increased the callus production character (43.4%) which was nearly equal to that of root development character (46.7%), it is mean that 92.9 percent of the calli had differentiated roots and it could become more efficient if more of the rooted calli could be induced to form shoots.

This result agreement with cold treatment, which was reported by Achar, (2002), Showed in Cabbage, a chilling treatment of 24 hrs. at 4 ^{o}C at the start of culture period resulted in a higher embryo yield compared to a treatment of 48 hrs. and Nada *et al*., (2003), they found that 24hrs. at 4 ^{o}C to be best in broccoli anther culture for the callugenic frequency percentage. The callugenic frequency percentage increased to 60.8% under cold treatment, while under room temperature was 22.5%. It is means that, the cold treatment of 24hrs. at 4 ^{o}C was the most effective for inducing embryogenesis in

cabbage and produced better results for callus formation and root differentiation in broccoli.

The interaction effect between genotype and temperature on callus formation, rooting percentage and root development of Broccoli:

Combined data in Table (3) showed significant effect of interaction between genotype and temperature treatment on callus induction, rooting. percentage and root development characters.

Concerning callus production character, results in Table (3), indicated that, although Landmark hybrid gave the highest value (52.1%) under room temperature as compared with the other hybrids, it gave the same value (52.1%) under chilling treatment. It is means that, the relationship without significant between the genotype Landmark and low temperature. On the other hand, although Landmark and Dome hybrids had nearly equal values (52.1 and 52.6%), respectively at low temperature, but the genotypic effect was completely different between them under control condition (52.1 and 19.7%), respectively. The cold pretreatment exhibited (166.6%) over that room temperature for Dome hybrid. It is clear that, the best relationship was found between the genotype Dome and buds chilling. Although pinnacle hybrid gave the lowest value (13.6%) under room temperature as compared with the other hybrids, the cold treatment effect reached (28.5%) and exhibited (109.2%) over that room temperature, Table (3). It is indicated that, a clear relationship was obtained between the genotype pinnacle with respect to their effect on callus induction and cold treatment. In the meantime, Although, Sunrise hybrid gave the highest value (68%) under cold treatment, it gave the lowest value for the chilling treatment exhibition (35.1%) only over that room temperature as compared with pinnacle (109.2%) and Dome (166.6%) hybrids, Table (3). Similar results agreement have been reported by Modyaeva , (1990), with callus formation in cabbage, who found that cold treatment effective one line only from anthers of 11 lines and 8 varieties were cultured. And also agreement with Nada *et al,* (2003), they mentioned that, the chilling treatment increased the callugenic frequency percentage from anther culture of broccoli by (473.1, 216.4, 207.9, 93.4 and 82.5%) in the pinnacle, Landmark, Dome, Sunrise and Comanche hybrids, respectively compared with the control.

Table (3) revealed that the rooting percentage character varied from genotype to another in each of the two temperature treatments. Although, Sunrise hybrid gave the highest values under two temperature treatments the cold treatment exhibited (56.1%) only over that control. In the meantime, although, Dome hybrid gave the lowest value (1.33%) at room temperature, the cold pretreatment exhibited the highest value (490.2%) over

that control condition as compared with the other hybrids. It is indicated that, the best relationship was showed between the genotype Dome and buds chilling. In the same Table (3), although, Landmark gave the higher values under two temperature treatments than Pinnacle and Comanche, the low temperature exhibited the lowest value (41.2%) over that room temperature as compared with pinnacle (184.8%) and Comanche (147.8%), respectively.

Table(3): Interaction between F1 hybrids and temperature treatments on callus formation and root development of anther culture in Broccoli.

F1 hyb.	Temp.	No. of anther culture	Callus formation %	Increase % over Rt	Rooting percentage %	Increase % over Rt	Root development %	Increase % over Rt
Pinnacle	Rt	285	13.60	-	2.10	-	15.38	_
	Lt	285	28.45	109.2	5.98	184.8	20.99	36.5
Comanche	Rt	219	22.90	-	2.30	_	10.0	_
	Lt	209	26.25	14.6	5.70	147.8	21.82	118.2
Dome	Rt	76	19.74	-	1.33	_	6.70	-
	Lt	76	52.63	166.6	7.85	490.2	15.00	123.9
Landmark	Rt	538	52.05	-	5.95	_	11.43	-
	Lt	535	52.14	0.2	8.40	41.2	16.13	41.1
Sunrise	Rt	225	50.32	-	16.53	_	32.74	_
	Lt	225	68.00	35.1	25.80	56.1	37.91	15.8
LSD (0.05)	-	-	0.84	-	0.28	-	1.23	-

Rt = Room temperature , Lt = Low temperature ,

From the result in Table (3), the interaction was obtained between the five genotypes and the two temperature treatments for root development character. Although, sunrise hybrid gave the highest values under two temperature treatments, the chilling treatment exhibited the lowest value (15.8%) over that control as compared with the other tested hybrids. On the other hand, although Dome hybrid gave the lowest values in each of the two temperature treatments, the cold treatment exhibited the highest value (123.9%) over that control condition as compared with the other tested hybrids, Table (3).

From the results of the present study, it might be concluded that, considerable variation in response to anther culture was obvious among genotypes within the same variety. Some of these variations could be attributed to genetic differences or related to physiological conditions.

Conclusion

Pretreatment of flower buds after sterilization at low temperature (4°C for 24 hrs) as reported in this work with B.oleraceae L.var. italica could be considered as an additional type of beneficial stress treatment. Low temperature increased the yield of anther produced each of callus and root also the callus developed root characters .This treatment might be specific and had not been previously studied with Brassica oleraceae L.var. italica especially in Egypt. Therefore , it is believable that this factor is considered to be very important to increase the callus production, there is an evidence to suggest that the plants regenerated after slight callusing can be utilized to obtain somaclonal variants. In addition, calli developed from anthers culture and the differentiation of roots in callus, it could become more efficient if more of the rooted calli could be induced to form shoots, it expected that if these shoots are derived from single cells or cell clusters, they may possess variability to be exploited for agronomic improvement of this crop. Furthermore establishment of fast growing root can provide an excellent source of test material for breeding stocks from root cuttings in vitro and for the regeneration of shoots from root segments.

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العلاقة بين التركيب الوراثي ودرجة الحرارة المنخفضة على إنتاج الكالس ونمو الجذور الناتج من زراعة متك البروكلي

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الملخص العربي

أجري هذا البحث خلال الفترة من ٢٠٠٦ حتى ٢٠٠٧ بغرض دراسة تأثير تعريض البراعم الزهرية المعقمة لدرجة الحرارة المنخفضة (٤°م لمدة ٢٤ ساعة) على تكشف الكالس الناتج من زراعة المتك لخمس هجن بروكلي.

تمت زراعة خمس هجن بروكلي وهي (سانريس ودوم من هولندا) و (لاندمارك وبنسل من أمريكا) و(كومانش من اليابان) بأرض بحوث الخضر بالدقي بمعهد بحوث البساتين في ٢٠٠٦/٩/٢٠، وتم جمع البراعم الزهرية من أربع نباتات منتخبة من كل هجين خلال الفترة من نهاية ديسمبر ٢٠٠٦ حتى نهاية يناير ٢٠٠٧ وتعقيمها ثم تعريضها لدرجة الحرارة المنخفضة (٤°م لمدة ٢٤ ساعة) وتم زراعة المتك الناتج منها بمعمل زراعة الأنسجة ببحوث الخضر بالدقي وكذلك تمت زراعة متك معاملة المقارنة بعد تعقيم البراعم تحت درجة حرارة الغرفة وكانت البيئة المستخدمة للزراعة هي بيئة (ميلر ١٩٦٣) مضاف إليها (٢ ملليجرام/لتر) لكل من أندول اسيتك اسد و ٢–٤ داى كلوروفينوكس اسيتك اسد مع (١ ملليجرام) لكل من الكينتين ومستخلص الخميرة بغرض إنتاج الكالس. وتم استبعاد الـ ٢–٤ داى كلوروفيتوكس اسيتك اسد وخفض كل من الكينتين وأندول اسيتك اسد إلى (٠.٥ ملليجرام/لتر) من البيئة بغرض تكشف الكالس.

وأخذت البيانات على الصفات التالية:

١– استجابة المتك لإنتاج الكالس بعد أربع أسابيع من الزراعة.

٢– استجابة المتك لإنتاج الجذور بعد تكشف الكالس خلال ثمان أسابيع من بداية تكشف الكالس.

٣– استجابة الكالس لنمو الجذور خلال ثمان أسابيع من بداية تكشف الكالس.

وأوضحت النتائج أن تعريض البراعم الزهرية المعقمة لدرجة الحرارة المنخفضة (٤°م لمدة ٢٤ ساعة) أدت إلى زيادة نسبة استجابة المتك لإنتاج الكالس من ٣١.٧% إلى ٤٥.٥% أي بزيادة قدرها ٤٣.٤% مقارنة بالزراعة تحت درجة حرارة الغرفة، وكذلك زيادة نسبة استجابة المتك لإنتاج الجذور من ٥.٦% إلى ١٠.٦% أي بزيادة قدرها ٩٠.٦% مقارنة بالزراعة تحت درجة حرارة الغرفة وكذلك زيادة نسبة استجابة الكالس لنمو الجذور من ١٥.٣% إلى ٢٢.٤% أي بزيادة قدرها ٤٦.٧% مقارنة بالزراعة تحت درجة حرارة الغرفة. بالإضافة إلى وجود اختلافات معنوية بين هجن البروكلي في الصفات الثلاثة المدروسة ويرجع ذلك إلى الاختلاف في التراكيب الوراثية بينهم فقد أعطى الهجين سانريس أعلى نسبة للصفات الثلاثة وهي ٥٩.٢% لصفة استجابة المتك لإنتاج الكالس، ٢١.٢% لصفة استجابة المتك لإنتاج الجذور وأخيراً ٣٥.٣% لصفة استجابة الكالس لنمو الجذور. في حين أعطى الهجين بنسل أقل نسبة ٢١.٠٣% لصفة استجابة المتك لإنتاج الكالس والهجين كومانش ٤.٠% لصفة استجابة المتك لإنتاج الجذور والهجين دوم ٨.٦% لصفة استجابة الكالس لنمو الجذور.

وأوضحت النتائج أيضاً أن الهجين سانريس أعطى أعلى استجابة للصفات الثلاثة المدروسة سواء تحت ظروف الحرارة المنخفضة أو درجة حرارة الغرفة بينما الهجين دوم كان أفضل الهجن في الاستجابة لتأثير درجة الحرارة المنخفضة (٤°م لمدة ٢٤ ساعة) للصفات الثلاثة المدروسة.

ولذلك فأن تعريض براعم البروكلي المعقمة لدرجة الحرارة المنخفضة (٤°م لمدة ٢٤ ساعة) يؤدي إلى زيادة استجابة المتك لإنتاج الكالس ونمو الجذور وكذلك تكشف الكالس الناتج من زراعة متك هذه البراعم وأن التركيب الوراثي له تأثير ايجابي وهام في إنتاج الكالس وتكوين ونمو الجذور الناتج من زراعة متك البروكلي.