

RECOVERY OF HYBRID EMBRYOS OBTAINED FROM INTERSPECIFIC CROSSES BETWEEN COMMON AND TEPARY BEAN

By

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ABSTRACT

Interspecific crosses were made between two common bean cultivars: Giza 3 and Contender, and a tepary bean line: Tepary 13. Pod, embryo, and callus culture techniques were used to recover the hybrid embryos of the interspecific crosses. The immature hybrid pods and immature embryos gave the best results when cultured on medium contained MS mineral nutrients with KNO_3 and NH_4NO_3 deleted and 10 mM KCl and 1.5 mM K_2SO_4 added, 20 g sucrose, 100 mg myo-inositol, 10 mg thiamin. HCl, 1 mg nicotinic acid, 1 mg pyridoxine. HCl, 3 mg glycine, 40 mM glutamine, 20 mM asparagine, and 1 g casein hydrolysate per liter. The cultured immature hybrid pods increased in weight by 72.6% and 34.4% and in length by 12.1% and 10.7% after 28 days from culturing for the crosses Giza 3 x Tepary 13 and Contender x Tepary 13, respectively. The cultured hybrid embryos of the cross Contender x Tepary 13 significantly preceded those of the cross Giza 3 x Tepary 13 concerning forming regular shoots with chlorophyll as well as normal roots. The hybrid embryo-derived callus gave the best results concerning propagation and proliferation when cultured on medium contained macro- and micro- elements of Murashige and Skoog medium, 100 mg/l myo-inositol, 0.4 mg/l thiamin.HCl, 1 mg/l nicotinic acid, 1 mg/l pyridoxine.HCl, 4 mg/l kinetin, 2% sucrose, and 0.8% agar.

INTRODUCTION

Water shortage in many parts of the world is expecting to be the limiting factor for agriculture in the near future. The demand for drought-resistant cultivars is increasing due to the limiting water sources which are not going to be enough for irrigation of crops, especially with the continuous increase of population.

Interspecific hybridization:

Common bean (*Phaseolus vulgaris*) is one of the most popular vegetable crops in Egypt. Unfortunately, common bean is susceptible to drought. On the other hand the tepary bean (*Phaseolus acutifolius*) is drought tolerant and adopted to dry-land culture (Mok *et al.*, 1978; Thomas *et al.*, 1983; Pratt *et al.*, 1985; Mok *et al.*, 1986; Andrade-Anguilar and Jackson, 1988; Kumar *et al.*, 1988; Angelini and Allavena, 1989; Mohamed, 1990; Sabja *et al.*, 1990; Mohamed *et al.* 1992). Transfer of drought tolerance trait from *P. acutifolius* to *P. vulgaris* will be of great importance. The interspecific hybridization between the common and tepary beans has been proposed to facilitate genetic exchange between these two *Phaseolus* species. The restricted development of hybrid embryos, low

embryo survival in *in vitro* culture and during acclimatization, abnormal development of interspecific plants and the low fertility of these plants are considered the major barriers for the success of such interspecific hybridization (Mohamed, 1990).

Thomas *et al.* (1983), Mok *et al.* (1986), Andrade-Angular and Jackson (1988), Mohamed (1990) and Sabja *et al.* (1990) reported the importance of transferring drought resistance from the tepary bean (*P. acutifolius*) to the common bean (*P. vulgaris*) through interspecific crosses between the two species using the common bean as the female parent and they were successful in obtaining F₁ hybrids from these crosses by *in vitro* embryo culture technique. Mohamed (1990), who performed six interspecific crosses among three genotypes of *P. vulgaris* (as female parents) and two genotypes of *P. acutifolius* (as male parents) found that the crosses differed greatly in pod and seed set percent, seed weight, and total number of seeds having an excisable hybrid embryos. Andrade-Angular and Jackson (1988) mentioned that the success of interspecific hybridization between *P. vulgaris* and *P. acutifolius* depended upon the pollination technique, the species and individual genotypes used as female and male parents and the growth conditions. A high hybridization efficiency was achieved when *P. vulgaris* was used as female parent. The growth of the hybrid was determinate like the female parent, *P. vulgaris*, but leaf morphology was closer to the male parent, *P. acutifolius*. McElory (1985) derived a *P. vulgaris* line (Xan-159) from a fertile cross between *P. vulgaris* and *P. acutifolius* and backcross to the common bean parent.

Pod culture:

Culture of immature pods was found to be a good methodology to solve the problem of the restricted development of hybrid embryos obtained from the crosses between the common and tepary beans (Sabja *et al.*, 1990). They studied seed and embryo development in pods of the interspecific cross *P. vulgaris* X *P. acutifolius* cultured on specific medium and found that the weight of seeds and length of embryos increased significantly during culture. However, usually only one or occasionally two seeds located at the middle portions of the pod developed to maturity.

Embryo culture:

Embryo culture technique has been suggested to recover the interspecific hybrids between the tepary and common bean. Mohamed (1990) and Sabja *et al.* (1990) succeeded in obtaining excisable interspecific hybrid embryos from crosses between common and Tepary beans. The genotype of parents influenced the growth rate of cultured embryos obtained from crosses between common and tepary beans (Mok *et al.*, 1978; Pratt *et al.*, 1985; Mohamed, 1990; Sabja *et al.*, 1990). Also, stafford and Davies (1979) found that the genotype of the cultured pea embryos had a significant effect on the growth rate the embryos.

Mok *et al.* (1986) mentioned that, although fertilization in crosses between tepary and common bean occurred and embryos were formed, the interspecific hybrid embryos were generally limited in their developmental potential. The particular stage at which the developmental arrest of interspecific hybrid embryos occurs, were found to be dependent on the interspecific combination (e.g. the genotype of the common and tepary bean parents) as well as the direction of the cross (Sabja *et al.*, 1990).

RECOVERY OF HYBRID EMBRYOS

Stafford and Dariver (1979) stated that kinitin and other cytokinins were not essential in the culture medium for the growth of the immature pea (*Pisum sativum*) embryos. On the other hand Mohamed (1990) found that cytokinins played an important role in the normal growth of the cultured hybrid embryos obtained from crosses between some common and tepary bean germplasm. Hassan (1991) reported that the culture medium with relatively high concentration of auxin and relatively low concentration of cytokinin will encourage callus formation of the cultured explant.

Sucrose content were found to have a positive effect on the performance of the cultured pea embryos due to its role in adjusting the osmotic pressure of the culture medium (Stafford and Davies, 1987).

Callus culture:

Kumar *et al.* (1988) induced an embryogenic callus and regenerated plants from cell suspension culture of leaf-derived callus in *P. acutifolius* and observed an increase in the regeneration rate on media containing cytokinin. Mohamed *et al.* (1992) indicated the importance of including the cytokinin (1 mg benzyladenine/L) in the culture medium of leaf pedicel-derived callus of common bean to obtain large number of shoot primordia. On the other hand, Angelini and Allavena (1989) and Angelini *et al.* (1990) reported the difficulty of regenerating *P. vulgaris* plantlets from callus culture.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm and Tissue Culture Laboratory of the Department of Horticulture; Faculty of Agriculture, Moshtohor, Zagazig University, during the successive summer seasons of 1991, 1992, 1993 and 1994.

Interspecific hybridization:

Two common bean cultivars: Giza 3 and Contender, and a tepary bean line: Tepary 13, were used in this study. The two common bean cultivars are used on a large scale in Egypt and are characterized by lack of drought resistance. Seeds of the tepary line used in this study were kindly provided by Dr. D.P. Coyne; University of Nebraska-Lincoln; U.S.A. This Tepary line is characterized by resistance to drought.

Plants of each cultivar or line were previously selfed for two generations during summers of 1991 and 1992.

The seeds were planted in the experimental farm on March 15, 1993. Crosses were made between each of the common bean cultivars and the tepary bean line using the common bean cultivars as the female parents. The very limited number of interspecific hybrid pods, obtained after pollinating many flower buds of Giza 3 and Contender with pollen grains taken from Tepary 13, were harvested at the dry stage. Then, seeds were obtained from the dry pods and kept to the next season.

On March 15, 1994, interspecific hybrid seeds of both crosses: Giza 3 X Tepary 13 and Contender X Tepary 13, and seeds of parents: Giza 3, Contender and Tepary 13 were planted in the field. At full blooming stage ten flower buds from each genotype were tagged. Pollen grains were obtained from anthers of the tagged flower buds for each genotype just after their anthesis in order to examine the viability of the pollen grains using the method described by Kitat (1959).

Crosses were repeated in this season of 1994 between common bean cultivars and the tepary bean line using the common bean cultivars as the female parents. Hybrid pods of 14 days old - as suggested by Sabja *et al.* (1990)- were picked up from the plants and sent to the Tissue Culture Laboratory. The immature pods were surface sterilized in 70% ethanol for 30 seconds, and then in 35% clorox (1.8% sodium hypochloride) for 10 minutes. Thereafter, the immature pods were rinsed in sterile distilled water several times and dried using sterile filter papers. After that, the immature pods became ready for the purpose of pod and embryo culture.

Pod culture:

Each sterilized immature pod was placed in 2.5x20 cm tube filled with 10 ml of liquid medium developed by Barratt (1986). The culture medium contained the following ingredients: MS mineral nutrients (Murashige and Skoog, 1962) with KNO₃ and NH₄NO₃ deleted and 10 mM KCl and 1.5 mM K₂SO₄ added; 20 g/l sucrose; 100 mg/l myo-inositol; 10 mg/l thiamine.HCl; 1 mg/l nicotinic acid; 1 mg/l pyridoxine.HCl; 3 mg/l glycine; 40 mM/l glutamine; 20 mM/l asparagine, and 1 g/l casein hydrolysate. The pedicel of the immature pod was immersed in the liquid medium and the immature pod was supported upright by a bridge made from sterile filter paper. The tubes were sealed using parafilm and kept in a growth chamber under temperature of 26±2°C and photoperiod of 14 hrs for 28 days.

Weight and length of each immature pods were recorded before and after culturing. Percentage of weight and length increase of cultured immature pods were calculated using the following formula:

$$\text{Weight increase \%} = \frac{\text{Weight of immature pod after culturing} - \text{Weight of immature pod before culturing}}{\text{Weight of immature pod before culturing}} \times 100$$

$$\text{Length increase \%} = \frac{\text{Length of immature pod after culturing} - \text{Length of immature pod before culturing}}{\text{Length of immature pod before culturing}} \times 100$$

The experimental design used in conducting this experiment was randomized complete block design with four replicates. Each replicate contained 10 culture tubes for each interspecific hybrid.

Embryo culture:

Hybrid embryos were excised from the sterilized immature pods under aseptic conditions. Hybrid embryos of each interspecific cross were cultured on the following culture media:

Medium 1 (Barratt, 1986).

This medium contained the following ingredients: MS- mineral nutrients (Murashige and Skoog, 1962) with KNO₃ and NH₄NO₃ deleted and 10 mM KCl and 1-5mM K₂SO₄ added; 20 g/l sucrose; 100 mg/l myo-inositol; 10 mg/l thiamine.HCl; 1 mg/l nicotinic acid; 1 mg/l pyridoxine.HCl; 3 mg/l Glycine, 40 mM/l Glutamine, 20 mM/l asparagine, and 1 g/l casein hydrolysate. The pH of the medium was adjusted to 5.7 before autoclaving at 120°C under 1.2 kg/cm² for 20 minutes.

Medium 2 (Stafford and Davies, 1979).

RECOVERY OF HYBRID EMBRYOS

The following ingredients were used in preparing this medium: the macro- and micro-elements of Murashige and Skoog (1962); a vitamin solution (myo-inositol, 100 mg/l; thiamine.HCl, 0.1 mg/l; pyridoxin.HCl, 0.5 mg/l; nicotinic acid, 0.5 mg/l); iron as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (27.9 mg/l) and disodium EDTA (37.5 mg/l). The carbohydrate source was sucrose 2% and was solidified with 0.8% Bacto Difco Agar. A modification has been made for the contents of this medium by adding 40 mM/l glutamine; 20 mM/l asparagine; 0.5 mg/l IBA and 0.1 mg/l kinetin. The pH of the medium was adjusted to 5.0 before autoclaving as previously mentioned.

Medium 3 (Mohamed, 1990).

This medium contained the following ingredients: MS- mineral nutrients (Murashige and Skoog, 1962); 40 g/l sucrose; 100 mg/l myo-inositol; 10 mg/l thiamin; 1 mg/l nicotinic acid; 1 mg/l pyridoxin; 1 mg/l kinetin and was solidified with 0.8% Bacto Difco Agar. The pH of the medium was adjusted to 5.7 before autoclaving as mentioned before.

The culture tubes/jars were kept after sealing with parafilm in the growth chamber under temperature of $26 \pm 2^\circ\text{C}$ and photoperiod of 14 hrs for 28 days.

The experimental design used in conducting this experiment was split-plot design with three replicates. The culture media were assigned to the main plots and the genotypes of the interspecific hybrids were assigned to the sub-plots.

The following characteristics were recorded as follows:

$$1\text{- Callus formation \%} = \frac{\text{No. of embryos which formed callus}}{\text{Total no. of cultured embryos}} \times 100$$

$$2\text{- Shooting \%} = \frac{\text{No. of embryos which formed shoots}}{\text{Total no. of cultured embryos}} \times 100$$

$$3\text{- Rooting \%} = \frac{\text{No. of embryos which formed roots}}{\text{Total no. of cultured embryos}} \times 100$$

$$4\text{- Proliferation \%} = \frac{\text{No. of embryos which proliferated}}{\text{Total no. of cultured embryos}} \times 100$$

$$5\text{- Chlorophyll formation \%} = \frac{\text{No. of embryos with green shoots}}{\text{Total no. of cultured embryos}} \times 100$$

$$6\text{- Survival \%} = \frac{\text{No. of embryos which showed normal growth}}{\text{Total no. of cultured embryos}} \times 100$$

Callus culture:

The callus formed by the embryos of the cross Contender X Tepary 13 which were cultured on medium 2 (Stafford and Davies, 1979) were transformed under aseptic conditions to four different culture media "a", "b", "c" and "d". Each of the four culture media contained the following ingredients: macro- and micro-elements of Murashige and

Skoog (1962); 100 mg/l myo-insitol; 0.4 mg/l thiamin.HCl; 1 mg/l nicotinic acid and 1 mg/l pyridoxine.HCl. However, the kinetin content differed in the different culture media where medium A, B, C and D contained 1, 2, 3 and 4 mg kinetin/l, respectively. The carbohydrate source was sucrose 2% and was solidified with 0.8% Bacto Difco agar. The pH of the medium was adjusted to 5.7 before autoclaving at 120°C under 1.2 kg/cm² for 20 minutes.

The culture jars were kept in the growth chamber under temperature of 26±2°C and photoperiod of 14 hrs for 28 days. The experimental design used in conducting this experiment was a complete randomized block design with 3 replicates. The following measurements were quantitatively recorded as follows:

- No. of callus which showed increase in size
- 1- Callus growth % = $\frac{\text{Total no. of cultured callus}}{\text{No. of callus which formed shoots}} \times 100$
- 2- Shooting % = $\frac{\text{Total no. of cultured callus}}{\text{No. of callus which formed roots}} \times 100$
- 3- Rooting % = $\frac{\text{Total no. of cultured callus}}{\text{No. of callus which proliferated}} \times 100$
- 4- Proliferation % = $\frac{\text{Total no. of cultured callus}}{\text{No. of shoots with green color}} \times 100$
- 5- Chlorophyll formation % = $\frac{\text{Total no. of cultured callus}}{\text{No. of callus which showed normal growth}} \times 100$
- 6- Survival % = $\frac{\text{Total no. of cultured callus}}{\text{No. of callus which showed normal growth}} \times 100$

In all experiments, data recorded as percentages were transformed using the arcsine transformation as described by Steel and Torrie (1980). Analysis of variance was performed using the method described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Interspecific hybridization:

Percentage of pod set were very low for the crosses made in summer of 1993, e.g. Giza 3 X tepary 13 (0.67%) and Contender X Tepary 13 (0.86%) (Table, 1).

Table (1): Number of pollinated flower buds, number and percentage of pod set, and total number of seeds for the crosses Giza 3 X Tepary 13 and Contender X Tepary 13.

Cross	No. of pollinated flower buds	Pod set		Total number of obtained seeds
		No.	%	
Giza 3 X Tepary 13	450	3	0.67	8
Contender X Tepary 13	348	3	0.86	6

RECOVERY OF HYBRID EMBRYOS

Reduction in leaf area of the F_1 plants as compared with those of their female parents (common beans) was observed for the crosses Giza 3 X Tepary 13 and Contender X Tepary 13 (Fig., 1). This result agreed with what observed by Andrade-Anguilar and Jackson (1988) who mentioned that the leaf morphology of the interspecific hybrid obtained from crosses between common and tepary beans was closer to the male parent *P. acutifolius*. Indeterminate growth habit was transferred from Tepary 13 to the F_1 plants of both crosses (Fig., 2). This result is different from what was found by Andrade-Anguilar and Jackson (1988) who found that the growth of the hybrid obtained from crosses between *P. vulgaris* and *P. acutifolius* was determinate like the female parent, *P. vulgaris*. Such difference could be due to the effect of genotype involved. Flower color of F_1 plants of both crosses was different from the parental cultivars/line (Fig., 3). These observations indicated that the obtained hybrid seeds were resulted from hybridization between the common and tepary beans and not from selfing and/or contamination of female parent flowers.

Plants of the interspecific hybrids (F_1) failed in pod setting. This could be due to the observed high percentage of pollen grains sterility (Table, 2 and Fig., 4). Percentages of the viable pollen grains obtained from hybrid plants (F_1) of the crosses Giza 3 X Tepary 13 and Contender x Tepary 13 were 2.3% and 4.4%, respectively. The failure of interspecific hybrid plants (F_1) in pod setting in spite of the existence of small percentage of viable pollen grains (Table, 2) could be due to the scarcity of these viable pollen grains, and/or to self-incompatibility. The low fertility of the F_1 plants obtained from the crosses between common and tepary beans has been reported (Mohamed, 1990). Making backcross of such hybrid plants to both parents is suggested to solve the problem of pod setting failure. A *P. vulgaris* line (Xan-159) was derived from a fertile cross between *P. vulgaris* and *P. acutifolius* and backcross to the common bean parent by McElory (1985). Table (2): Percentage of viable and unviable pollen grains of parents and interspecific hybrids between the common and Tepary beans.

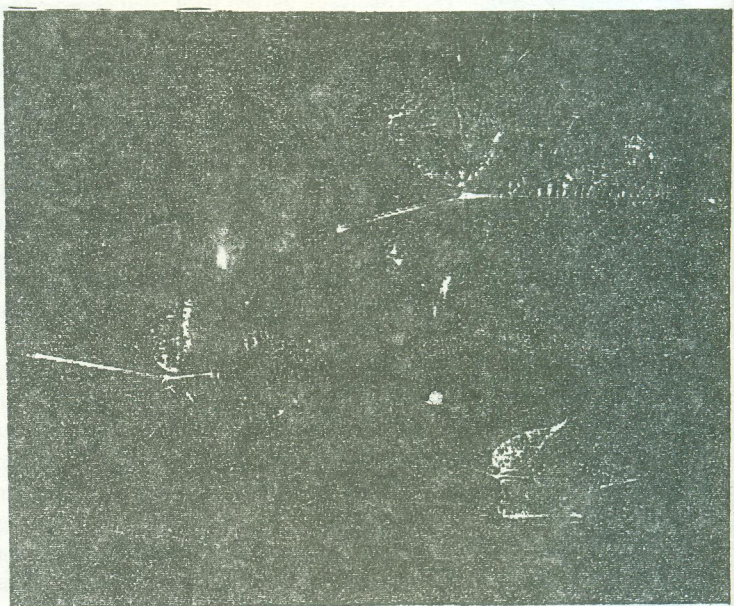
Population	Percentage of pollen grains		t value
	Viable	Unviable	
Giza 3	78.6	21.4	10.59**
Contender	77.8	22.2	10.26**
Tepary 13	78.9	21.1	20.85**
F1: Giza 3 X Tepary 13	2.3	97.7	3.04*
F2: Contender X Tepary 13	4.4	95.6	4.70**

* Significant at 5% level of significance.

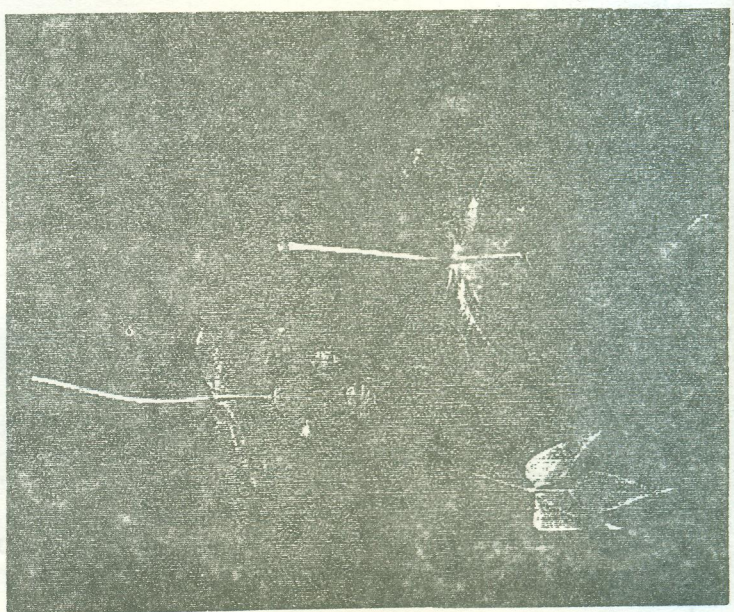
** Significant at 1% level of significance.

Pod culture:

Results in Table (3) show a significant increase in pod weight after 28 days from pod culture for the interspecific cross between Giza 3 and Tepary 13 where the percentage of weight increase were 72.6. However, in case of the interspecific cross between Contender and Tepary 13 the cultured pods were increased only by 34.4%. In addition, cultured pods of the crosses: Giza 3 X Tepary 13 and Contender X Tepary 13 were increased in length after 28 days from culturing (Table, 3). Pods of the cross Giza 3 X



1



2

Fig. (1): Leaves of the parents and F_1 plants of the crosses between Giza-3 X Tepary 13 (1) and Contender X Tepary 13 (2). Reduction in leaf area of F_1 plants was observed in both crosses (1 and 2).

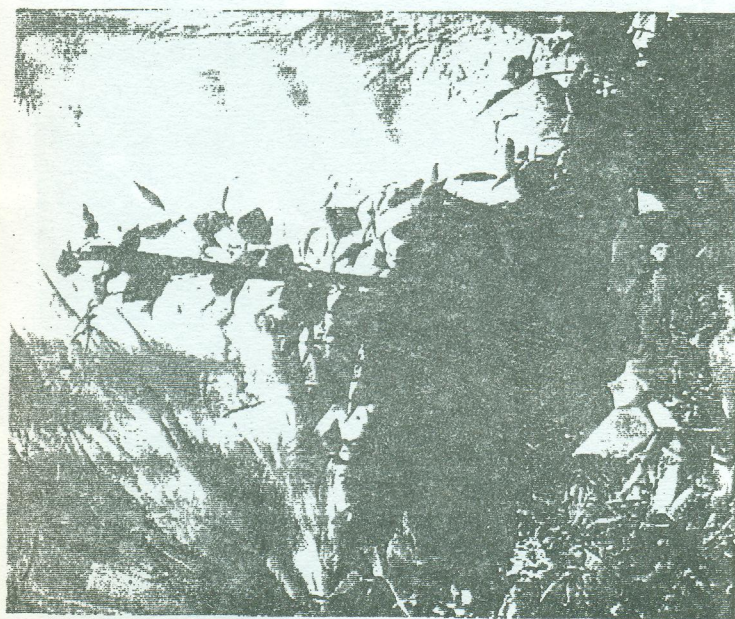


Fig. (2): F₁ plants of the interspecific crosses between (1) Giza-3 X Tepary 13 and (2) Contender X Tepary 13 Indeterminate growth habit, transferred from Tepary 13, showed complete dominance.

RECOVERY OF HYBRID EMBRYOS

(1990) and Sabja *et al.* (1990). In addition, embryos of the different cultivars/lines of pea gave different growth rates when cultured on a certain medium (Stafford and Davies, 1979).

Embryos cultured on medium 2 did not form shoots or roots but they developed only undifferentiated callus tissue (Table, 4 and Fig., 7). This could be due to the relatively high concentration of IBA (0.5 mg/l) and low concentration of kinitin (0.1 mg/l) contained in medium 2. These results are in agreement with what has been reported by Hassan (1991), who mentioned that the culture medium with relatively high concentration of auxin and relatively low concentration of cytokinin will enhance callus formation of the cultured explant. These results are also in agreement with those of Mohamed (1990), who found that kinitin was not a strong enough cytokinin to encourage the cultured hybrid embryos of the interspecific crosses between common and tepary beans to form normal shoots and roots.

Even though, having normal embryo growth was the objective of culturing the immature embryos of the interspecific bean crosses on the different culture media, the callus formation by the immature embryos cultured on medium 2 (Table, 4) can still be considered a success because the cells of the callus tissues have the same genotype of these cultured embryos. By using cell culture technique, hundreds of the individual cells which can be obtained from the formed callus tissue will have the potential of forming too many plantlets with the same genotype of the cultured embryo. Obtaining large number of plantlets from an individual immature hybrid embryo will be of great value. However, research is still needed to study the potential of using the cell culture technique with beans. A success has been achieved in tepary bean by Kumar *et al.* (1988) who regenerated tepary bean plants from callus cultures.

Callus culture:

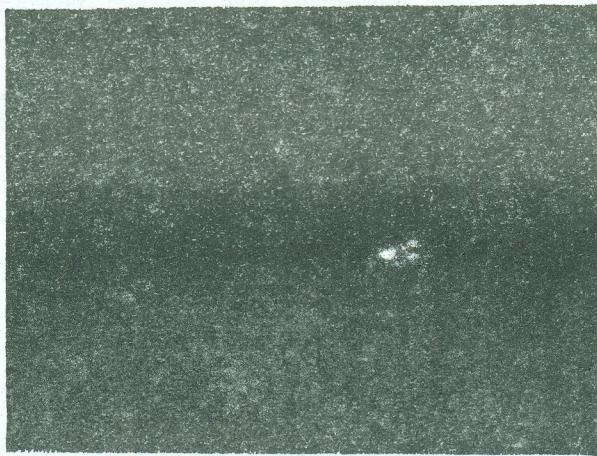
The hybrid embryo-derived callus of the cross between common bean (cv. Contender) and tepary bean (Line 13) showed no significant differences concerning the percentages of shoots, root and chlorophyll formation when cultured on the different types of medium 2 which had different concentrations of kinitin (Tables, 5 and Fig., 7). However, percentages of cultured embryo-derived callus which increased in size and proliferated differed significantly with the different used culture media. The highest percentage of embryo-derived callus which increased in size was associated with the callus cultured on medium D (63.08%) followed by callus cultured on medium C (48.67%), medium B (38.00%) and medium A (23.04%). In a descending order, the highest percentage of cultured embryo-derived callus which proliferated was detected in case of culturing the callus on medium D (72.59%) followed by medium C (58.25%), medium B (40.33%) and medium A (28.08%). Based on these results medium D can be considered the best medium for the propagation and proliferation of the hybrid embryo-derived callus (Fig., 7). This could be due to the relatively high concentration of kinitin (4 mg/l). The importance of involving cytokinins in the culture medium for normal shoot morphogenesis and of cultured callus has been reported in case of culturing the leaf pedicel-derived callus of common bean (Mohamed *et al.*, 1992). An increase in the regeneration rate from culturing cell suspension derived from leaf callus of *P. acutifolius* on medium containing

Table (5): Effect of different modified media on some aspects of cultured hybrid embryo-derived callus of the cross between Contender and Tepary13 line.

Modified medium	Kinetin mg/l	Callus growth (%)	Survival (%)	Shooting (%)	Rooting (%)	Chlorophyll (%)	Proliferation (%)
A	1	23.04	76.92	78.93	19.11	78.93	28.08
B	2	38.00	90.00	71.78	32.97	71.78	40.33
C	3	48.67	90.00	72.57	23.85	72.57	58.25
D	4	63.08	90.00	83.10	26.27	83.10	72.59
L.S.D. at 0.05		25.68	N.S.	N.S.	N.S.	N.S.	14.98



1



2



3

Fig. (7): Hybrid embryo-derived callus of the interspecific cross between cv. Contender and line Tepary 13 (1) 21 days, (2) 28 days and (3) 35 days after culturing on medium D.

cytokinins was observed by Kumar *et al.* (1988). On the other hand, Angelini and Allavena (1989) and Angelini *et al.* (1990) reported that it was difficult to regenerate *P. vulgaris* plants from callus cultures.

In conclusion, pod and embryo culture techniques could be very useful in solving the problem of the restricted development of hybrid embryos which usually occurs after making crosses between common and tepary beans. These techniques will ease the process of transferring the drought resistance genes from the tepary bean lines to common bean cultivars through hybridization.

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RECOVERY OF HYBRID EMBRYOS

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**الحصول على أجنة دجينية ناتجة من المهن النوعية
بين الطاسوليا العادية والطاسوليا التيباري**

أحمد رضا عجمي
 أنجي أبو النصر أبو سدرة
 محمد السعيد زكي
 لطفي عبدالفتاح عبدالرحمن
 قسم النباتات = كلية الزراعة بمشهور = جامعة الزقازيق (الرغ بها) - مصر.

تم عمل هجن نوعية بين صنفين من أصناف الفاصوليا العادية هما "جزء ٣" و"كونتندر" وسلالة من فاصوليا القباري هي "تباري ١٣"، وقد استخدمت طرق زراعة القرون، زراعة الأجنة، زراعة الكالس، وذلك لإنجاح عملية الحصول على أجنة هجينة من هذه الهجن النوعية. أعطت القرون الهجينة الغير ناضجة والأجنة الهجينة الغير ناضجة أحسن النتائج عند زراعتها على بيئة تحقوي على أملاح بيئة "موراشيچ وسكوج" مع استخدام بون ٣، ن بدء، ن ١ وإضافة ١٠ مليمول بون كل، ١،٥ مليمول بون ٢ كل أ، بالإضافة إلى ٢٠ جم سكروز، ٢٠٠ جم ميونيوسيتول، ١٠ جم ثيامين بد كل، ١ جم حمض النيكوتينك، ١ جم بيروثوكسين بد كل، ٣ جم جلايسين، ٤٠ مليمول جلوتامين، ٢٠ مليمول أسبارجين، ١ جم كازين هيدروكسيلات/لتر.

حدثت زيادة في وزن القرون الهجينة الغير ناضجة مقدارها ٧٧,٦ ٪، ٣٤,٤ ٪ وزيادة في الطول مقدارها ١٧,١ ٪، ١٠,٧٧ ٪ بعد ٢٨ يوما من الزراعة وذلك بالنسبة للهجين جيزة ٣ (أ) * تيارى ١٣ (أب) وكونتدر (أم) * تيارى ١٣ (أب) على الترتيب.

تفاوتت الأجنة الهجينة المفزعة والنتيجة من التهجين كونتدر (أم) * تيارى ١٣ (أب) على تلك الناتجة من التهجين جيزة ٣ (أم) * تيارى ١٣ (أب) وذلك بالنسبة لتكوين نموات خضرية وحذور عادية.

أعطى الكاس الناتج من الأجنة الهجينية أفضل النتائج بالنسبة للكتاف والتميز عند زراعته على بيئة أملاح بيئة موراشيج وسكوج^٤ بالإضافة إلى ١٠٠ مجم/لتر موراينوسيتول، ٤ مجم/لتر ثيامين، يد كل، ١ مجم/لتر حمض بيكوتيك، ١ مجم/لتر بيرودوكسين، يد كل، ٢ مجم/لتر كينتين، ٠,٨ ٪ أجار.