THE EFFECT OF *IN VITRO* CULTURE OF IMMATURE SUNFLOWER EMBRYOS ON SOME MORPHOLOGICAL AND AGRONOMICAL CHARACTERS

Christophe PELLETIER, Denis TOURVIEILLE & Félicity VEAR

INRA - Centre de Recherches Agronomiques de Clermont-Ferrand

Domaine de Crouëlle. F - 63039 - Clermont-Ferrand Cédex. France.

SUMMARY

The technique of *in vitro* culture of immature sunflower embryos is widely used to accelerate breeding programmes. It is therefore important to determine its effects on the main characters selected during inbred line fixation. For four genotypes, a comparison was made between plants obtained from *in vitro* culture of immature embryos plante out in the field, and those from seeds sown in the usual way.

A constant reduction of 30 % was observed for plant height. Capitulum and stem diameters were also reduced, but to variable extent (by -3 to -9 % and -10 to + 16% respectively).
Effects on 1000-seed weight, seed number per head and oil content were also determined. Two of the genotypes were resistant to *Phomopsis* and two susceptible. The plants obtained from *in vitro* culture showed the same distinction of reponse to leaf infections with *Phomopsis* mycelium as those grown from seed.

INTRODUCTION

In vitro culture (c.i.v.) of immature embryos is used in sunflowers (*Helianthus annuus*) to reduce the duration of a growth cycle from 90 - 120 days to 60-70 days (ALISSA et al, 1986). In breeding programmes it can be used to accelerate back-crossing programmes to introduce major genes or cytoplasmic male sterility (CHANDLER and BEARD, 1983).

It would also be of great interest to be able to use immature embryo culture in pedigree selection programmes for inbred lines. However, it is necessary to be sure that the selection process remains efficient when plants obtained from c.i.v. of immature embryos are judged. ASPIROZ et al (1987) showed significant

reductions in plant height, capitulum diameter and leaf size. PELLETIER (1988) found no gain in resistance to Phomopsis (*Diaporthe helianthi*) using tests on F3 and F4 plants grown in pots in the greenhouse after immature embryo culture. In a preliminary study, MIRMAN (1989) observed significant effects of c.i.v. of immature embryos on many agronomic characters, with changes in the classification of genotypes for flowering date, weight of 1000 seed and oil content, but not for maturity date, seed number and *Sclerotinia* and Phomopsis resistance.

This paper reports a comparison between plants sown normally in the field and those obtained from c.i.v. of immature embryos, for the main agronomic characters, and in particular resistance to Phomopsis, since it appeared of particular interest to accelerate the breeding of lines resistant to this recently appeared disease.

MATERIALS and METHODS

Genotypes :

Four inbred lines, coded PH1, PH2, PH3 and PH4, were chosen for their differing reactions to *Diaporthe helianthi* , the first two were known to be susceptible, the two latter, resistant.

Growing Conditions :

To obtain plants from *in vitro* culture of immature embryos, the 4 lines were sown at several dates in the greenhouse and selfed under paper bags. Embryos were harvested 10 to 12 days after the beginning of flowering and for all the genotypes at the same time.

The embryos were first grown on a MURASHIGE and SKOOG (1962) medium modified by ALISSA et al (1986) and containing hormones. After 2 days they were then transfered onto a medium with the same composition but lacking hormones. After about 10 days, the plantlets were pricked out in pots maintained in the greenhouse for another 10 days to condition them to more normal growth conditions (absence of 100% humidity). Fourty plants of each genotype were then planted out in the field.

To obtain plants from seed, at the same date as the harvesting of immature embryos, seeds of the four genotypes were sown in the field. After thinning at the 2 leaf stage, 40 plants of each genotype were retained.

Morphological and agronomic observations :

Phomopsis test : When the plants showed flower buds 5 to 8cm in diameter, 3 young, fully grown leaves of each were infected with *Diaporthe* mycelium by the method described by TOURVIEILLE et al. (1988). An agar explant containing the mycelium was placed on the extremity of the main vein, with the fungus in contact with the leaf. To prevent drying, it was covered with aluminium foil stapled in place. Twice daily irrigations were provided by sprinklers for 20 days. Observations were then made of the lengths of Phomopsis necroses along the main veins of the leaves.

Flowering date was noted, then at the onset of maturity, **capitulum** and **stem diameters** and **plant height** were measured. After harvest, **seed number, weight of 1000 seed** and **oil content** were determined, the latter by Nuclear Magnetic Resonance.

RESULTS

The results are presented in Table 1. There were significant differences between genotypes for all characters.

Morphological characters :

Stem diameter : Plants obtained from c.i.v. generally showed slightly thinner stems, although this was not the case for PH3, which showed a significant increase in stem diameter (+ 16%).

Capitulum diameter : Embryo culture appears to have little effect on this character, there was a significant reduction only for PH1.

Leaf number : There was a general reduction in leaf number of plants obtained by c.i.v., significant for all genotypes except PH4.

Plant height : It is for this character that embryo culture had the greatest effect (- 30%), and all the genotypes reacted in the same way.

Agronomic characters :

Phomopsis reaction : The effect of embryo culture on lesion length was not marked. The relative reaction of the four genotypes did not vary according to treatment. In fact, there was a slightly better distinction between resistant and susceptible genotypes after embryo culture : the resistant inbreds (PH3, PH4) showed a decrease of 23% in lesion length, whereas the susceptibles (PH1, PH2) showed a 13% increase. For PH2 and PH4, these differences were significant.

Flowering date : *In vitro* embryo culture increased earliness for all genotypes although only to a small extent for PH4.

Seed number : Together with plant height, this was the character which was most affected by embryo culture. The increase in seed number for PH3 appears to be an anomally.

Weight 1000 seed : This character is the opposite of all the others, with increases for PH2, PH3 and PH4.

Oil content : The effect of embryo culture varied according to genotype, with an increase for PH1 and PH3 and a reduction for PH2 and PH4. This character showed the greatest change in genotype classification between field-sown seed and embryo culture, PH2 normaaly having the highest oil content, but showing the lowest after c.i.v.

DISCUSSION

There is a general reduction in plant vigour after embryo culture, but this effect becomes reduced with time : of the 4 morphological characters observed, capitulum diameter, determined after flowering, shows the least effect. The large effect on height, which confirms that of ASPIROZ et al (1987), may perhaps be linked with the reduced number of leaves and greater earliness of flowering in a hypothesis that immature embryo culture gives a shortened vegetative development. With only one exception (PH3, stem diameter), genotypic classification is not altered by treatment, and selection for morphological characters on plants from embryo culture would be possible. This contrasts with MIRMAN (1989) who observed changes in the relative heights of 11 genotypes when subjected to the same treatments. This could depend on the reaction of individual genotypes to c.i.v.

Embryo culture generally causes a reduction in the value of agronomic characters, related to the reduced vigour. For the weight of 1000 seed, the increases following embryo culture may be related to the small number of seed developing, such that there was no competition between seeds. The much earlier flowering date of plants obtained from embryo culture may be due to premature aging, but could also result from the fact that, during the first 20 days, the immature embryos and plantlets were grown at 23°C, whereas the seeds in the field had a mean temperature not exceeding 15°C. Nevertheless, genotype classification remained the same for the 2 treatments.

Characteristics concerning seed showed more variability in genotype classification, with the anomalous seed number of PH3 and the reduced 1000 seed weight of PH1. In particular, oil content was extremely variable, and it does not appear possible to select for oil content on plants obtained from c.i.v. This is in agreement with the results of MIRMAN (1989).

In contrast, it appears quite possible to determine resistance to Phomopsis on plants obtained from c.i.v. of immature embryos. Resistant and susceptible genotypes are easily distinguished, in agreement with MIRMAN (1989). However the slight to moderate reduction in lesion length for the resistant genotypes after embryo culture may explain the inefficiency of selection by PELLETIER (1988). For a genotype like PH4, if progeny of a plant with 3.0 cm lesions were obtained by c.i.v. and those plants with lesions of 2.4 cm selected, the apparent gain in resistance would not necessarily be genetic.

At present, it is difficult to explain the different reactions of resistant and susceptible genotypes in response to embryo culture. It must be concluded that distinction , for example in F2 progenies, between highly resistant and susceptible material is possible on plants obtained from embryo culture, but choice between plants with small differences in resistance must be subject to caution.

Concerning genotype adaptation to embryo culture, it may be noted that, except for Phomopsis lesions, PH4 shows the smallest effects. Selection using this technique may thus be of variable interest according to the gentic origin of the material studied.

Table 1 : Observations of morphological and agronomic characters of 4 sunflower genotypes grown from seed or produced by *in vitro* culture of immature embryos.
* SD : Seed, EM : Embryos, % : Change of EM compared with SD, LSD : Least Significant Difference

		Stem diameter	Capitulum diameter	Leaf number	Plant height	Phomopsis reaction	Flowering date	Seed number	Weight 1000 seed	Oil content
PH1	**SD***	21.7	16.2	28.4	123.9	5.5	74.2	564.4	67.2	45.3
	EM*	19.6	14.8	22.9	84.3	5.9	65.3	460.5	54.0	48.1
	%*	-10	-9	-20	-32	+6	-12	-18	-20	+6
PH2	**SD**	16.7	15.0	20.0	71.6	7.1	72.8	501.7	54.0	50.4
	EM	15.8	14.3	16.5	48.2	8.6	63.1	256.8	83.5	42.8
	%	-5	-4	-18	-33	+20	-13	-49	+55	-15
PH3	**SD**	16.4	11.8	17.9	101.4	2.7	72.7	244.1	33.9	43.6
	EM	19.0	11.4	14.7	73.5	2.2	61.5	337.7	43.9	46.1
	%	+16	-3	-18	-28	-17	-15	+38	+30	+5
PH4	**SD**	23.3	18.8	22.7	148.4	3.3	79.6	856	40.6	49.1
	EM	22.7	18.4	22.0	105.6	2.4	76.1	580	45.0	46.3
	%	-2	-2	-3	-29	-29	-4	-32	+11	-6
genotype	F	66.6	90.3	418	604.6	199.3	522	74	266.2	24
LSD*		1.5	1.1	1	5.4	0.8	1.1	86.8	4.4	1.7

REFERENCES

ALISSA A., JONARD R., SERIEYS H., VINCOURT P., 1986. La culture d'embryons isolés *in vitro* dans un programme d'amélioration du tournesol. Compte Rendu Académie des Sciences Paris, t.302 Série III, n°5, 161-164.

AZPIROZ H.S., VINCOURT P., SERIEYS H., GALLAIS A., 1987. La culture *in vitro* des embryons immatures dans l'accélération du cycle de sélection des lignées de tournesol et ses effets morphovégétatifs. Helia, n°10, 35-38.

CHANDLER J.M., BEARD B.H., 1983. Embryo culture of *Helianthus* hybrids. Crop Science, 23, 1004-1008.

MIRMAN N., 1989. Utilisation de la culture d'embryon immature pour la sélection des caractères agronomiques et tentative d'androgènèse chez le tournesol cultivé. Mémoire de D.E.A., Ecole Nationale Supérieure Féminine d'Agronomie RENNES, I.N.R.A., 50 pages.

MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15, 473-497.

PELLETIER C., 1988. Etude de l'utilisation de la culture d'embryons immatures *in vitro* pour la résistance au Phomopsis du tournesol. Ménoire de D.E.A., Uni. CLERMONT-FERRAND II, INRA, 35 pages.

TOURVIEILLE D., VEAR F., PELLETIER C., 1988. Use of two mycelium tests in breeding sunflower resistant to phomopsis. In Proceedings of the 12 th International Sunflower Conference. Novi Sad, Yugoslavia, July 25 to 29, Vol II, 110-114