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SUNFLOWER TISSUE CULTURE AND USE IN SELECTION FOR RESISTANCE TO PHOMA MACDONALDII AND WHITE MOLD (SCLEROTINIA SCLEROTIORUM).

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SUMMARY

Of the 50 sunflower inbreds tested for regeneration ability in cell culture, nine produced embryogenic type callus. Of these nine, four produced plantlets, H55, HA300, Rassvet, and HA89*3/*H. rigidus*. This tissue culture regeneration system was implemented for study of two disease systems. In the first system, sunflower calli were grown on second stage regeneration medium containing culture filtrates of *Phoma macdonaldii*, the causal agent of Phoma black stem and a causal organism of premature death of sunflower in North Dakota. Sigco 475, a resistant hybrid, showed resistance on filtrate media, while HA300 and R025 were susceptible on filtrate media. In the second system, three isolates of *Sclerotinia sclerotiorum*, the causal agent of white mold, were tested for oxalic acid (a putative toxin) production. The isolates showed a correlation between oxalic acid production and virulence. Three hybrids Northrup King 277, Northrup King 285, and Interstate 894 showing varying field resistance to *Sclerotinia* have been selected and are being tested for disease reaction as callus cultures on media containing culture extracts of these isolates.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) regeneration from oil-type lines has been reported by several workers [Bohorova et al. 1985, Everett et al. 1987, Greco et al. 1984, Lupi et al. 1987, and Paterson and Everett 1985]. These systems use hypocotyl tissue and a one or two step protocol for regeneration of primary callus. Other systems use immature embryos, but these are not suitable for *in vitro* disease screening. Most of the hypocotyl callus culture systems involve regeneration of primary calli (not subcultured). Our objectives were 1) to expand the length of time in culture by several months or subcultures while retaining the ability to regenerate and 2) to use this expanded capacity to develop screening systems for studying disease resistance. We have had previous success using other disease systems [Hartman et al. 1984, Hartman et al. 1986, Hartman et al. 1987], and it was our intent to study the interactions of sunflower calli with *Phoma macdonaldii* Boerema and *Sclerotinia sclerotiorum* (Lib.) deBary.

This research on sunflower cell culture involved three studies. First, the ability of 50 inbreds to regenerate from cell culture was tested. Twenty progeny lines of the best regenerating line, H55, were then tested for heritability of regeneration. Second, hybrid lines Sigco 475 and Interstate 432, and inbred lines HA300 and R025 which showed different responses to Phoma in the greenhouse, [Donald et al. 1987] were tested *in vitro* using culture filtrate of the pathogen. Third, hybrid lines Northrup King 277, Interstate 894, and Northrup King 285 which show differential responses to *Sclerotinia* in the field are being tested for disease reaction *in vitro* using cell culture methods.

MATERIALS AND METHODS

Cell cultures for all three studies were initiated from 11-18 day old sunflower seedlings. Seeds were surface sterilized in 70% ethanol for 10 min, 40% bleach for 20 min and rinsed in two changes of sterile distilled water. Seeds were placed in 25 x 150 mm tubes containing MS medium [Murashige and Skoog 1962] and

incubated at 24 ± 1 C under 24 hr cool white fluorescent light ($20uEm^{-2}sec^{-1}$) Hypocotyl sections (2-3 mm) were aseptically dissected and placed on callus induction medium (CI) containing MS major and minor salts, MS iron stock, B5 vitamins, 500 mg casamino acids, 40 mg adenine sulfate, 0.1 mg giberellic acid (GA), 0.1 mg 2,4-dichlorophenoxyacetic acid (2,4-D), 30 g sucrose, and 8 g agar. Plates were incubated in the dark at 24 ± 1 C. After one month, callus was transferred to shoot induction (SI) medium containing the same formulation as CI except that 2,4-D was replaced with 1.0 mg benzylaminopurine (BAP). Plates were incubated at 24 ± 1 C and 16 hr light.

The inbreds tested in the regeneration study are listed in Table 1. Callus type was evaluated after growth on CI medium. Callus was labeled "embryogenic" or "nonembryogenic" type [Everett et al. 1987]. Callus of the embryogenic type was sometimes placed on a third medium (II) without BAP or 2,4-D, but containing indole acetic acid (IAA) at 0.1 mg to enhance growth of previously formed shoots.

P. macdonaldii cultures were initiated on potato dextrose agar (PDA) for 14 days. For toxic filtrate production, 5 mm^2 mycelial plugs were cut from the PDA and used to inoculate 1 liter Fernbach flasks containing 500 ml Czapeks-Dox broth and 45 ml sunflower extract. Sunflower extract was prepared by macerating 5.0 g of 2 week old sunflower seedlings in 90 ml of distilled water in a blender, and coarse filtered. Flasks were incubated on a shaker at room temperature for 3-4 weeks. The mycelial growth was removed by coarse filtration and the extract was filter sterilized prior to addition to the medium. Filtrate was added to SI medium at various concentrations for testing. Callus tissue from the resistant cultivar Sigco 475, and the cultivars RO25, 432, and HA300 were used in testing. Callus fresh weight on SI medium with and without filtrate was obtained after 30 days growth.

S. sclerotiorum filtrate was obtained by growing cultures for one wk on PDA, and then inoculating flasks containing 500 ml Potato Dextrose Broth. Cultures were incubated for one month on a shaker at room temperature. Filtrate from these flasks was collected after coarse filtration followed by filter sterilization. Filtrate was added to SI medium for screening studies. Three isolates of Sclerotinia, ND1, ND25 and ND37, obtained from B. D. Nelson, North Dakota State University, which had been previously rated low, moderate and highly virulent, were used in the trials. These isolates were evaluated by gas chromatography for oxalic acid production. Oxalic acid is a putative toxin produced by S. sclerotiorum. Hybrid cultivars NK277, IS894 and NK285 which had been previously rated as somewhat resistant, intermediate and susceptible respectively, are being used in this study. Callus tissue from these hybrids is being tested for disease reaction on medium containing Sclerotinia filtrate.

RESULTS

Of the 50 inbreds tested for the ability to produce callus tissue and regenerate, nine produced embryogenic type callus (Table 1). This callus is light brown to gray in color, nodular and slimey in consistency. Of the nine producing embryogenic callus, four produced plantlets, H55, HA300, Rassvet and HA89*3/H. rigidus. The most vigorous plantlets were from H55. Of the 20 H55 crosses tested for ability to form callus, all produced embryogenic type callus.

Table 2 shows the growth of sunflower callus tissue with and without Phoma culture filtrates added to the SI medium. Callus growth was reduced significantly by the addition of filtrate to the media for all lines tested. However, the resistant cultivar Sigco 475 grew significantly better in the presence of filtrate than HA300 or RO25, but not Sigco 432 (statistics not shown). No resistant plants have been regenerated from our Phoma selection system, but we

Table 1. Sunflower inbred lines tested for regeneration ability in cell culture.

Inbred Line	Source	Callus Induction CI medium	Embryogenic or Nonembryogenic	Plantlets formed
H55	So. Africa	+	E	+
HA300	USDA	+	E	+
HA99	USDA	+	E	-
RHA274	USDA	+	N	-
FL0305	Italy	+	N	-
R025	Italy	+	E	-
HA303	USDA	-	N	-
HA302	USDA	-	N	-
HA301	USDA	-	N	-
HA822	USDA	+	E	-
RHA278	USDA	+	N	-
MM-10	So. Africa	+	N	-
Pemir	Spain	+	E	-
H52	So. Africa	+	E	-
Felix	Romania	+	N	-
Pipa Blanca	Spain	+	N	-
Issanka	Spain	+	N	-
Arg	Argentina	+	N	-
RHA271	USDA	+	N	-
Progress	USSR	+	N	-
Donsky	Yugoslavia	+	N	-
RHA801	USDA	+	N	-
HA89*3/*H. rigidus*	USDA	+	E	+
HA89	USDA	+	N	-
Yugo 18	Yugoslavia	+	N	-
Rassvet	USSR	+	E	+
Chernjanka	USSR	+	N	-
Salyut	USSR	+	N	-
Start	USSR	+	N	-
Chakinski	USSR	+	N	-
Avangard	USSR	+	N	-
Majak	USSR	+	N	-
Armavirec	USSR	+	N	-
UNII6540	USSR	+	N	-
UNII8931	USSR	+	N	-
Smena	USSR	+	N	-
CM587	Canada	+	N	-
LSR(2)	So. Africa	+	N	-
Comp	So. Africa	+	N	-
RK74-198-2	So. Africa	+	N	-
*H. rigidus**3/HA89	USDA	+	N	-
RK74-22-1	So. Africa	+	N	-
CM361	Canada	+	N	-
CM589	Canada	+	N	-
HA335	USDA	+	N	-
HA336	USDA	+	N	-
HA337	USDA	+	N	-
HA338	USDA	-	N	-
HA339	USDA	+	N	-
HA340	USDA	-	N	-

do have putative resistant calli from susceptible hybrid 432. Selection and regeneration of resistant calli from hybrid Sigco 432 and inbred HA300 will continue.

Testing of sunflower callus for response to filtrate from Sclerotinia has been recently initiated and is currently in progress. These experiments will follow the same protocol as a similar study using Sclerotinia filtrate and dry bean (Phaseolus vulgaris L.) that we recently completed [Hartman et al. 1987]. In this study, culture filtrates of Sclerotinia were incorporated into bean callus culture media. A correlation existed between growth chamber susceptibility/resistance of bean lines to Sclerotinia and reaction of the calli. The three Sclerotinia isolates produce different amounts of oxalic acid as determined by gas chromatography, ND1 the least, ND25 intermediate and ND37 the most oxalic acid. Bean calli from plants with some resistance showed resistance to filtrates from ND1 and ND25, but not ND37. Analysis by gas chromatography shows that resistant bean calli uptake less oxalic acid than susceptible. We hope to find similar results in the sunflower trials.

Table 2. Fresh weight (g) of calli[1] from four sunflower cultivars on shoot induction media (SI) with and without amendment by culture filtrates of P. macdonaldii.

	Sigco 475	432	R025
SI medium	3.30a[2]	2.44a	2.77a
SI medium + 125 ml/L filtrate	1.73b	1.47b	0.39b
SI medium + 150 ml/L filtrate	1.23b	1.24b	0.29b
	Sigco 475	HA300	R025
SI medium	2.54a	2.95a	2.79a
SI medium + 100 ml/L filtrate	1.92b	1.10b	0.38b

[1]Average 20 plates/treatment.

[2]Alpha = 0.05.

DISCUSSION

Approximately 14% of the inbred lines tested showed regeneration potential. However, accurate testing was difficult since ethylene production greatly effects sunflower regeneration. The plants from which the hypocotyls were derived were grown in test tubes. Contact of the leaves on test tube surface combined with the moisture in the tube often resulted in ethylene damage. Tissue affected in this way could not be accurately scored for regeneration potential.

Hypocotyl tissue from plants derived from H55 crosses all formed embryogenic like calli. This offers encouragement that the regeneration trait may be heritable and can be bred into other sunflower lines of interest.

Plants could be regenerated from tissue up to four mo. old. However, the regeneration percentage is low. We would like to increase our percentage so that our chance of recovery of disease resistant mutants will be increased. Further modifications in procedure and medium are no doubt necessary and will be implemented in the future.

No resistant plants have been regenerated from our Phoma selection system. However, we do have putative resistant calli from hybrid Sigco 432. Selections from Sigco 432 and inbred line HA300 will continue.

The sclerotinia work was very successful when using dry bean calli cultures. We will continue this work with the sunflower lines. The hybrid NK277 shows embryogenic like calli in culture. Even though NK277 is somewhat resistant to the white mold disease, we would like to increase that resistance by cell culture disease selection. We are encouraged that this line shows regeneration ability.

CONCLUSIONS

From this work, it appears that sunflower cell culture can be used to study and predict disease reactions of sunflower plants, and may be useful to isolate disease resistant mutants in screening trials.

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