PRELIMINARY RESULTS OF USING CELL CULTURE TO SCREEN FOR RESISTANCE TO PHOMOPSIS SP. (DIAPORTHE SP.) CAUSAL AGENT OF BROWN GRAY SPOT OF SUNFLOWER STEM

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SUMMARY

Four sunflower hybrids and one inbred line were evaluated for resistance to Phomopsis sp. (Diaporthe sp.) using cell culture. Dissected hypocotyls from 14 day old seedlings were placed on MS media with 2,4-D in the dark for one month and then grown in the light for one month on a second media containing BAP and culture filtrate from the fungus. A study was initiated to determine if resistance to Phomopsis (Diaporthe) could be identified by screening sunflower cell cultures with fungal filtrate. Calli of genotypes susceptible to Phomopsis were brown to black and grew slowly or not at all on culture filtrate media.

INTRODUCTION

Phomopsis sp. (Diaporthe sp.), the causal agent of gray brown spot of sunflower stem, was discovered for the first time in Yugoslavia in 1980 (Maric et al. 1980, Mihaljcevic et al. 1980). Since then it has been observed in Romania, Hungary, France, USA and several other countries. In 1987, we observed Phomopsis symptoms on sunflower in all of the important growing ares in the USA (Masirevic et al. 1988, in press).

Up to now, only a few resistant or tolerant sources of germplasm have been found, and these usually originated from wild Helianthus species. In order to identify additional resistant germplasm, an effective screening method is needed which will reliably distinguish susceptible and resistant germplasm. A greenhouse screening technique has been developed in which plants are sprayed with ascospores. This technique is hampered by the necessity of obtaining mature perithecia containing ascospores, which at the present time can only be produced on infected sunflower stems.

Tissue culture techniques may be useful in situations such as this where conventional screening techniques are not entirely satisfactory and not reliable in identifying resistant germplasm. In addition, tissue culture techniques may be able to take advantage of somaclonal variation or somatic cell fusion within a genotype and thus increase the chances of recovering resistant material.

Tissue culture studies in disease resistance have been successful in the past with sunflower and Phoma macdonaldii (Donald et al. 1986) and Sclerotinia sclerotiorum (Hartman et al. 1987), alfalfa (Medicago) and Fusarium oxysporum f. sp. medicaginis (Hartman et al. 1984), and dry bean (Phaesolus) and Pseudomonas syringae pv. phaseolicola (Hartman et al. 1986).

MATERIALS AND METHODS

Sunflower achenes were excised from the hulls of four hybrids: PAG/SG 103 and SEEDTEC 316 (both susceptible to Phomopsis), and NS-H-43 and NS-H-45 (both resistant to Phomopsis) and the USDA inbred line RHA 273 (susceptible). The kernels were surface sterilized in 70% ethanol for 15 min, followed by 25 min in 0.5% sodium hypochlorite, and then rinsed twice in sterile distilled water. The kernels were placed in Petri dishes containing B-5 media (Gamborg et al. 1968) with no hormones and incubated at 24 + 1 C under 25 hr light from cool white fluorescent bulbs (400 $uEm^{-2}sec^{-1}$). After 3 days the germinated seedlings were transferred to culture tubes (25x150 mm) with B-5 medium and grown for 12-17 days (not more than 20 days) (Paterson and Everett 1985) at 24 + 1 C and 24 hr photoperiod.

Hypocotyl sections (2-3 mm) were aseptically dissected from the seedlings and transferred to Petri dishes containing MS media (Murashige and Skoog 1962). This media contained MS major and minor salts, B5 vitamins, casamino acids (500 mg), adenine sulphate (40 mg), gibberellic acid (0.1 mg), 2,4-dichlorophenoxyacetic acid (0.1 mg), sucrose (30 g), and agar (8 g) per liter, with the pH adjusted to 5.8. Plates were incubated in the dark at 24 + 1 C. After 5 weeks (no more than 2 months), calli were subcultured onto modified MS media in which 2,4-D was replaced with 6-benzylaminopurine (BAP) at 1 mg/L, plus Phompsis culture filtrate at 200 and 400 ml/L using isolate SD (South Dakota). The filtrate was prepared by growing the fungus in shake culture for one month using Czapek's Dox broth plus a sunflower tissue extract (5 g freshly homogenized leaves/L). The shake culture was filter sterilized and added to the cooled, sterile, modified MS media. Calli from the different hybrids were grown for one mo at 24 + 1 C with a 24 hr photoperiod, after which they were inspected for viability and weighed.

RESULTS AND DISCUSSION

Hypocotyl explants grown on control media without filtrate formed calli of a light brown to gray color, while calli grown on media with filtrate showed reduced growth and browning after six days. After three weeks, calli of both susceptible and resistant genotypes were susceptible to the filtrate as evidenced by their dark brown color and lack of growth. There was a significant difference between calli on filtrate and calli on modified MS media without filtrate. Calli not on filtrate turned green, continued to grow and had significantly greater fresh weight. Sunflower tissue grown on modified MS media amended with Czapek's Dox media appeared similar to the control plates. It appears that the filtrate rates we used in our testing, 200 and 400 ml/L are toxic to both resistant and susceptible cultivars and lower rates are necessary to identify resistant germplasm. Research is continuing in this direction. It appears however, that the filtrate is active in vitro and is toxic to sunflower calli and can be used as a screening system for Phomopsis.

The ultimate goal is to identify existing sources of, or locate new sources of, resistant germplasm. It may be possible to find a green sector in otherwise susceptible callus, expanding the possibility of finding new sources of resistant germplasm. This possibility is encouraging because sunflower callus can be regenerated after prolonged exposure on screening systems.

REFERENCES

Donald P., C. Hartman, G. Secor. 1986. Use of cell culture to screen for resistance to Phoma macdonaldii. Sunflower Research Workshop. National Sunflower Association, Bismarck, ND, USA. 6 p.

Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Exp. Cell Res. 50:151.

Hartman, C. L., T. J. McCoy, and T. R. Knaus. 1984. Selection of alfalfa (Medicage sativa) cell lines and regeneration of plants resistant to the toxins produced by Fusarium oxysporum f. sp. medicaginis. Plant Sci. 34:183-184.

Hartman, C. L., G. A. Secor, J. R. Venette, and D. a. Albaugh. 1986. Response of bean calli to filtrate from Pseudomonas syringae pv. phaseolicola and correlation with whole plant disease reaction. Physiological and Molecular Plant Pathology 28:353-358.

Hartman, C. L., D. A. Albaugh, G. A. Secor, and J. R. Venette. 1987. Screening of dry bean calli to identify resistance to Sclerotinia sclerotiorum (Abst.) Phytopathology 77:(In press).

Marić, A., S. Maširević and S. Fayzalla. 1980. Pojava sive pegavosti stabla (Phomopsis sp.) do sada nepoznate bolesti suncokreta - Glasnik Zastite Bilja 12, Zagreb.

Mihaljčević, M., Petrov, M. Muntanjola-Cvetković, M. 1980. Phomopsis sp. novi parazit suncokreta u Jugoslaviji, Savremena polj. 11-12, Novi Sad.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologica Plantarum 15:473-497.

Paterson, K. E. and N. P. Everett. 1985. Regeneration of Helianthus annuus inbred plants from callus. Plant Sci. 42:125.