A PROPOSED SYSTEM FOR IDENTIFYING RACES OF SUNFLOWER RUST.

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Summary

As with many plant pathogens races occur within the rust (Puccinia helianthi) population. The number of races recognized by different workers varies from three to ten to a multitude. To facilitate the planning of breeding programmes and the international exchange of germplasm with effective rust resistance it would be most useful to have a standardized system for typing races of rust. To date only two genes R_1 and R_2 for resistance to rust have been described and lines containing each of them plus a 'universal suscept' are included in the differential set. Because rust reaction or infection types can be influenced by a whole range of climatic, host and pathogen factors, we suggest that all typing be conducted using the following conditions. i) Obtain single urediniospore isolates from each collection and multiply in isolation on a universal suscept to provide sufficient inoculum for the test. ii) Ensure that all differentials are true to type. iii) Grow seedlings of all differential lines in controlled environments with 18/22°C night/day temperatures and 600 μEm^{-2} sec^{-1} of light supplied in 12h photoperiods. iv) When the first true leaves are fully expanded inoculate them with about 500 fresh urediniospores cm^{-2}, spray with a fine mist of water and cover with plastic bags. v) Incubate for 16h in darkness at 20°C. vi) Remove plastic bags and replace plants in the controlled environment. vii) Inspect plants daily for reaction to rust and make a final rating 12 days after inoculation. viii) The rating system for reaction type is a modification of the numerical system used by Sackston (1962). The ratings are: 0, no visible reaction; 0;, fleck reaction; 1 small uredinia (<0.2 mm diameter) which sporulate weakly with flecking and chlorosis; 2 uredinia (⩾0.2 mm diameter) which sporulate freely with some chlorosis and 3 uredinia (>0.2 mm) which sporulate vigorously with little or no chlorosis. 0, 0; and 1 are resistant and 2 and 3 are susceptible reaction types. This system can be expanded as new information on resistance genes or reaction types becomes available.

Introduction

Rust (Puccinia helianthi Schw.) is an important disease in many sunflower growing areas of the world (Zimmer and Hoes, 1978) causing yield losses as high as 70% (Middleton and Obst, 1972; Brown et al., 1974). As with many diseases of broadacre crops, the most effective and economical method for farmers to control rust in sunflower is to grow rust resistant cultivars and there are breeding programmes in various parts of the world which have the development of rust resistance in cultivars as one of their objectives.

This resistance may be characterized by immunity to rust as expressed by the failure of the rust fungus to complete its uredinial infection cycle. In many crops this form of resistance is often conditioned by single dominant genes which are readily identified and manipulated, but it is often short-lived due to the development of new virulent strains of pathogen.

Physiologic specialization has been found in P. helianthi. Reports on the number of biologic forms, strains, race groups and races which have been recognized vary from three or four (Bailey, 1923; Brown, 1936; Sackston, 1962) to nine or ten (Hoes and Putt, 1962; Jabbar Miah et al., 1967). Hennessy and

Sackston (1972) stated that those races recognized by Hoes and Putt (1962) were not comparable to those recognized by Jabbar Miah et al. (1967). Furthermore, studies by Zimmer and Fick (1974) indicated that "a multitude of races of P. helianthi occur on the wild species". One race of rust had been recorded in Australian sunflower production areas before the identification of a second race in January 1983 (Kochman and Goulter, 1984). These correspond to races 1 and 3 respectively, in Sackston's (1962) system.

The methods of typing or identifying races of rust used by the various researchers cited in the previous paragraph have been described in their papers. The usual method is to inoculate a number of differential lines with urediniospores, incubate them and observe their reactions to infection. The variety of results obtained illustrate the heterogenous nature of virulence in P. helianthi but comparisons between the results of different workers is impossible because of the use of different differentiating lines and conditions which may modify rust development.

There were discussions on race identification of P. helianthi at a short meeting held in Australia during the 10th International Sunflower Conference. There was general agreement that some standardized system for identifying races would be most useful, particularly in facilitating the international exchange of germplasm with effective rust resistence and communication between rust researchers in various countries. Hence, in this paper we review some data published on differential lines and the factors which may effect rust development on them. We then propose a set of conditions to be used in a system of identifying races of P. helianthi.

Factors affecting rust development and reaction type

Disease development and expression (reaction type) are the product of interactions between pathogen, host and environmental factors. Variations in any of these factors can alter disease development or reaction type or both. Hence, during the typing of rust isolates, these factors should be considered and, where possible, standardized so that consistent, reproducable results can be obtained.

1) The pathogen.

Variability in pathogenicity of rust isolates will produce variable reaction types on sunflower lines. Several researchers already cited, used single uredinia in their studies of rust races. However, Dinoor et al. (1968) showed that two races of Puccinia coronata Cda. were able to form common uredia on susceptible oat cultivars and that virulence masked avirulence in these compound uredinia. Compound uredinia could occur in nature and if used in race typing could produce spurious combinations of reaction types on differential lines. In order to avoid this situation we suggest that type cultures of each race be established from single urediniospores and increased in isolation. These can then be used to check any anomolous reaction by other isolates on any differential.

2) The host - differential lines

Obviously the number of differential lines with different genes for resistance will govern the number of races which can be identified. If there are n genes for resistance, it is possible to identify 2^n races. Putt and Sackston (1963) described two genes for resistance (R_1 and R_2) which allowed the identification of four races. Jabbar Miah et al. (1967) stated that they had four

differentials with different resistance genes which would enable them to identify up to 16 races. However, it appears that some of these differential lines are very difficult to maintain true to type (Sackston pers. comm.) and we have only been able to obtain seed of lines containing the R_1 and R_2 genes. Even these lines were not homozygous for resistance when we received them and we obtained some curious reaction type results until we purified them.

Hence, despite the reports that four or more genes for resistance to P. helianthi occur, seed of lines containing them do not appear to be currently available from any source. Hence, we only have lines which are homozygous for R_1 and R_2. We would certainly appreciate information and seed of any other differential lines which other researchers have found to be homozygous for rust resistance and different to R_1 or R_2.

3) Environmental factors.

Dew period, temperature, light (duration and intensity) and inoculum density are all environmental factors which have been shown to affect the infection process and development of P. helianthi. Goulter (1983) found that at 20°C P. helianthi established infections and produced some uredinia after a 4h dew period with maximum numbers of uredinia produced after an 8 to 10h dew period. The number of uredinia produced on inoculated plants exposed to dew periods of 8, 10, 16, and 24h were not significantly different.

Sood and Sackston (1972) reported that temperatures for optimum germination and appressorium formation ranged from 10 to 25°C while the optimum temperature range for penetration was 15 to 25°C. Hennessy and Sackston (1970) reported that uredinia developed in 9 to 10 days at 20-22°C (night-day) and in 14 to 20 days at 10°C, while Kochman and Goulter (1982) reported that uredinia developed in 7-9 days at 18-22°C (night-day). Preliminary results in our laboratory indicate that rust development in some lines may be suppressed in a temperature regime of 25-32°C (night-day).

High light intensity during incubation can adversely affect germination, appressorium formation and penetration (Sood and Sackston, 1972). Moreover, it apears that low light intensity can be an important factor in P. helianthi development. Goulter et al. (1984) found that the reaction type produced by "race 1" on a hybrid cultivar carrying the R_1 gene for resistance changed with different light regimes. Under high light conditions (16h photoperiod and 400 $\mu Em^{-2}\ s^{-1}$ intensity) no uredinia were formed while under low light conditions (8h photoperiod and 400 $\mu Em^{-2}\ s^{-1}$) uredinia with diameters ranging from 0.185 to 0.214 mm, developed.

The density of urediniospores applied during inoculation can also affect development of P. helianthi on sunflower. Goulter et al. (1984) reported that generation times and diameter of uredinia were significantly reduced with increasing inoculum densities. Too high densities will induce necrotic reaction even in susceptible lines.

By using controlled environment cabinets and spore settling towers, all these factors can be standardized and conditions which are most favourable for rust development can be selected.

Classification of reaction types

Sackston (1962) described a numerical classification scheme for rating reaction types produced by P. helianthi on sunflowers, which has been widely used by

other researchers. However, we have encountered some difficulties with this scheme, particularly with the 2, 3 classifications which delineate resistant and susceptible reaction types, because the description of pustule size and vigour of sporulation is rather subjective. Hence, we propose that this sytem be modified with the following rating classifications. O, No visible reaction. O;, Fleck reaction. 1, Small uredinia (<0.2 mm in diameter) in association with flecking and chlorosis. 2, Uredinia (>0.2 mm diameter) which sporulate freely in association with chlorosis. 3, Uredinia (>0.2 mm diameter) which sporulate freely with little or no chlorosis. O, O; and 1 are resistant and 2 and 3 are susceptible reaction types.

Nomenclature of races

Currently there is little difficulty with nomenclature of races as there are only a few and these are classified as races 1, 2, etc. However, there is already one anomaly where race 2 is virulent on differentials with the R_2 gene, but race 3 is virulent on the R_1 gene. If there is an increase in the number of differential lines and races a more logical method of race nomenclature may be required. We suggest that, as with race nomenclature used for cereal rusts (Luig, 1983) and Phytophthora infestans (Mont.) de Bary (Black, 1952), that all differentials could be numbered and races could be identified numerically depending on their virulence on each differential. Hence, an isolate virulent only on the first differential would be classified as race 1, an isolate virulent on the second, third and sixth differential would be classified as race 2, 3, 6 and so on.

The differential containing the R_1 gene would be differential 1 and the R_2 gene differential 2. Hence the four races identified by Sackston (1962) would be classified as follows: Race 1 which is not virulent on either R_1 or R_2 would become race O, race 2 would remain as race 2, race 3 would become race 1 and race 4 would become race 1, 2.

Once a new differential line is established by a researcher, all relevant details of its parentage, mode of inheritance of resistence and details of reaction to previously identified races should be published in an internationally abstracted journal. Seed of any such differential should be increased and made available to anyone requiring it.

Conclusion - a proposed system for identifying races

Having considered all these factors which can lead to variability in reaction type, we propose that the following conditions be adopted and used in a standardized system for identifying races of P. helianthi.

i) Obtain single urediniospore isolates from each collection and multiply in isolation to provide sufficient inoculum for the test. This is to minimize variation in the pathogen. ii) Ensure that all differentials lines are true to type. iii) Grow seedlings of all differential lines in controlled environments with 18/22°C night-day temperatures and 600 μEm^{-2} sec^{-1} of light supplied in 12h photoperiods. iv) When the first true leaves are fully expanded, inoculate them with about 500 fresh urediniospores cm^{-2}, spray with a fine mist of water and cover with plastic bags. Spore settling towers (Brown and Kochman, 1973) can be used to obtain uniform inoculum deposition at the required density. v) Incubate for 16h in darkness at 20°C. vi) Remove plastic bags and replace plants in the controlled environment. vii) Inspect plants daily for reaction to rust and make

a final rating 12 days after inoculation. viii) The rating system for reaction type is a modification of the numerical system used by Sackston (1962).

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