Phenotypic selection improves the resistance of 'Mattenklee' red clover to Southern Anthracnose (*Colletotrichum trifolii*)

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Abstract

Southern Anthracnose, caused by *Colletotrichum trifolii*, is a serious disease of red clover (*Trifolium pratense*) and can lead to death of the plants. The degree of resistance is highly variable among cultivars. The objective of this study was to assess whether phenotypic selection within a cultivar improves resistance to Southern Anthracnose. From each of seven cultivars with moderate to low resistance level, we selected 10 resistant genotypes. Within each cultivar, the resistant genotypes were crossed by manual pollination. The resulting F_1 progeny of each cross was then evaluated in the greenhouse for resistance to *C. trifolii*, along with the original cultivars. Plants of the F_1 progeny survived at a rate of 53 to 77%, with a mean of 64%. The resistance of six out of seven cultivars was significantly improved after one cycle of selection. On average, the resistance level was increased by 28% (with a range of 18 to 52%) compared to the parental cultivar. The increase was highest in cultivars with a low initial resistance. The data suggest that few major genes are involved in Anthracnose resistance in the cultivars tested.

Keywords: *Trifolium pratense*, *Colletotrichum trifolii*, resistance, breeding

Introduction

Red clover (*Trifolium pratense* L.) is a high yielding, perennial forage legume, used mainly for cutting in pure stands or grass-clover mixtures and is often found in permanent grassland (Boller *et al.,* 2010). Due to its ability to fix atmospheric nitrogen, red clover has a high content of protein and is one of the most important forage legumes of temperate climates (Taylor and Quesenberry, 1996). Red clover is allogamous and has a strong gametophytic self-incompatibility system (Boller *et al.,* 2010). Swiss 'Mattenklee' (meadow clover) is an early flowering and persistent red clover type, which has been bred in Switzerland since the beginning of the 20^{th} century based on locally adapted landraces.

In the early 1990s a fungal disease of red clover, Southern Anthracnose (*Colletotrichum trifolii*), was observed for the first time in Switzerland (Boller *et al.,* 1998). Plants of the Swiss 'Mattenklee' cultivars proved to be susceptible to this disease. *Colletotrichum trifolii* spreads rapidly with conidia under warm and humid weather conditions. The fungus causes light to dark brown lesions predominantly on stems and petioles. These injuries can girdle the whole stem very quickly so that leaves or whole shoots dry out and die, resulting in significant yield losses. On the diseased tissue, black fruiting bodies (acervuli) with conidia and bristles (setae) are formed. The fungus also attacks the upper part of the taproot and the plant base, which in most cases leads to premature death of the whole plant. In addition to red clover, Southern Anthracnose can infect other host plants like alfalfa (*Medicago sativa*) and other *Medicago* and *Trifolium* species.

The most economically efficient way to control Southern Anthracnose is through breeding of resistant varieties. In the United States, red clover was successfully bred for resistance to *C. trifolii* as early as the 1950s (Taylor, 2008). The objectives of this paper were (1) to show if resistance to *C. trifolii* is inherited in the Swiss 'Mattenklee' red clover and (2) to search for resistant genotypes in this plant material in order to develop new resistant and persistent red clover cultivars.

Materials and methods

Inoculum preparation

Cultures of eight single-spore isolates of *C. trifolii* were obtained from diseased red clover plants collected in Ellighausen (Switzerland). The cultures were grown on PDA for 14 days at 18 °C under 12 h ultraviolet light irradiation. The inoculum consisted of an equal part of each of the eight isolates and was prepared by scraping spores from the agar plates. The spores were diluted in distilled water containing two drops of Tween 20 l^{-1}. Spore concentration was adjusted to 3×10^6 spores ml^{-1}.

Evaluation of resistance

Red clover plants were grown in a greenhouse at a day/night temperature of 22/20 °C and a 16 hour photoperiod. Seedlings were planted in boxes (0.3×0.4 m) filled with cultivation substrate at a plant to plant distance of 4 cm. Each cultivar or progeny of a cross was grown in a randomized complete block design with three to four replicates (depending on the number of seeds available). Each replicate consisted of up to 40 plants. Five-week old plants were cut about 2-3 cm above ground and the number of plants was assessed. Two weeks after cutting, the plants were sprayed with the inoculum until the plants were dripping wet. Plants were then covered with a transparent polyethylene foil for five days. Two, six and ten weeks after inoculation plants were cut for the second, third and fourth time, respectively. Three days after the last cut the surviving (resistant) plants of each cultivar or progeny were counted. Resistance was expressed as percent of surviving plants to the number of plants prior to inoculation.

Plant material

Out of seven cultivars (with a moderate to low resistance level) 8 resistant plants per cultivar were selected. Within each cultivar 4 biparental crosses were made by manual pollination (subject to assumption that self-fertilization does not occur in our plant material). The resulting F_1 progeny of each of the 28 biparental crosses was then evaluated for resistance to *C. trifolii*, at the same time as the original cultivars.

Statistical analysis

Mann Whitney U-test was carried out for comparing the data of the cultivar and the F_1 progeny population. The observed ratios of resistant to susceptible plants in the F_1 populations were compared to theoretical ratio using Chi-square tests.

Results and discussion

Swiss 'Mattenklee' cultivars showed a low to moderate resistance to Swiss isolates of *C. trifolii*. Depending on the cultivar, they exhibited 15 to 50% resistance as determined by the percent of plant survival (Figure 1). The cultivars Merula and Pavo (both released in 2002) had the highest level of resistance. Surprisingly, the old varieties Renova and Rüttinova (released in 1964 and 1984, respectively) showed a comparable high percentage of surviving plants.

In the F_1 populations from crosses between resistant genotypes of identical 'Mattenklee' cultivars, plants survived at a rate of 53 to 77%, with a mean of 64%. The resistance of six out of seven cultivars was already significantly improved after just one cycle of selection. On average, the resistance level increased by 28% (with a range of 18 to 52%) compared to the parental cultivar. The increase was highest in Formica, a cultivar with a low initial resistance.

Only 9 out of 28 biparental crosses fitted a segregation ratio of 3:1 (resistant : susceptible) ($P>0.05$) and no progeny showed 100% resistant genotypes. The significant increase in the resistance level after one cycle of selection suggests that major effect resistance genes are involved in the cultivars tested.

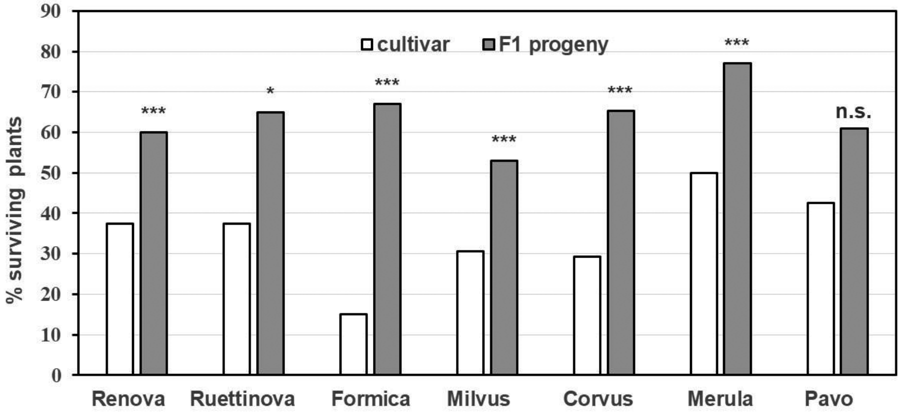

Figure 1. Resistance level, determined by percentage of surviving genotypes after an inoculation with *C. trifolii*, of seven Swiss 'Mattenklee' cultivars and the corresponding F_1 populations of crosses between resistant genotypes; * $P<0.05$, *** $P<0.01$.

Additionally, the segregation ratios in progeny populations from pair crosses between resistant genotypes indicate that more than one resistance gene is present.

Our data is in accordance with the observations of Jacob *et al.* (2015), where Pavo and Merula were among the most resistant cultivars tested, although at a much higher resistance level. Moreover, in both studies all tested cultivars showed improved resistance after just one cycle of recurrent selection.

Conclusion

The moderate resistance level of most Swiss 'Mattenklee' cultivars clearly indicates that at least one resistance gene, which confers resistance against *C. trifolii*, must be present in these cultivars. Moreover, the results show that the inoculation method described in this paper can be used to deploy resistance genes of old cultivars in breeding new resistant and persistent cultivars.

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