Field Crops Research 154 (2013) 195–200

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Assessment of diversity for resistance to spot blotch disease and its association with certain phenotypic traits in barely

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ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form 13 August 2013
Accepted 14 August 2013

Keywords:
Hordeum vulgare
Cochliobolus sativus
Leaf angle
Anthocyanin
Waxiness

ABSTRACT

A set of 1662 barley accessions from India, ICARDA and CIMMYT were evaluated over three cropping seasons for reaction to spot blotch (causative agent *Cochliobolus sativus*) infection, along with the four phenotypic traits waxiness, anthocyanin pigmentation, plant height and leaf angle. Only 5% of the entries showed any substantial resistance, while 31% were moderately resistant, 40% moderately susceptible and 24% fully susceptible. The range in mean area under disease progress curve (AUDPC) percent days and days to maturity of the best-performing 25 entries was 250–463 and 88–111, respectively, and most out-performed the best check entry. Four crosses were made between one of the resistant entries (EMBSN-27-4-1, BCU 570, BCU 455 and HMBSN-47-1) and one of the susceptible ones (RD 2503, RD 2624, RD 2614 and CIHO 3510). The F_3 and F_4 generations were used to test for genetic linkage between spot blotch reaction and the four phenotypic traits. Both waxiness and narrow leaf angle were positively associated with resistance, but neither plant height nor anthocyanin pigmentation was.

1. Introduction

Barley ranks fourth among the cereals after rice, wheat and maize (FAOSTAT, 2009); its importance reflects its broad adaptation, its suitability for both human consumption and animal feed and its unique brewing properties (Poehlman, 1985). A recognized feature of the crop is its ability to tolerate drought better than the other leading cereals (Guo et al., 2009). Like all crop species, barley suffers from various diseases, one of the most damaging of which is spot blotch (causative pathogen *Cochliobolus sativus*) (Kumar et al., 2007; Chand et al., 2008). The disease affects the leaf, sheath and stem (Chand et al., 2008), and in severe attacks, the fungus also infects the ear and causes shrivelling of the grain (Kiesling, 1985) and the formation of a black point at the embryo end (Chand et al., 2008). Global losses in grain yield can reach 33% (Clark, 1979), but in addition, the disease can compromise the malting quality of the grain and thus reduce the farmer's economic return (Nutter et al., 1985).

Although fungicide application can control spot blotch (Kiesling, 1985), its use increases the cost of cultivation and is considered to be environmentally hazardous. Frequent use of the same fungicide can rapidly lead to the development of fungicide resistance in the pathogen population (Golembiewski et al., 1995). Host resistance, just as it is for other diseases, is therefore widely considered to be the most sustainable method for combating spot blotch (Wilcoxson et al., 1990). Various attempts have been made to identify resistance donors (Jalli and Robinson, 2000) and to look for associations between resistance and readily selectable traits with which the resistance is associated (Joshi et al., 2004). In wheat, which is attacked by the same pathogen, plant height, the number of days to reach maturity (Joshi et al., 2002), leaf angle (Joshi and Chand, 2002), leaf tip necrosis (Joshi et al., 2004) and the stay green trait (Joshi et al., 2007a) have all been associated to spot blotch resistance. The formation of platelet-like wax particles on the surface of its leaves has been associated with quite high levels of resistance (Das et al., 1999). The present investigation was undertaken to characterize what genetic variation for resistance to spot blotch was harboured in a large *ex situ* barley germplasm collection, and to determine whether any association with resistance could be observed with waxiness, anthocyanin pigmentation, plant height and leaf angle. This information will be of significant help to breeders and wheat researchers in developing countries where conventional breeding is of paramount importance.

2. Materials and methods

2.1. Evaluation of spot blotch resistance

The germplasm set comprised 1662 accessions, assembled from collections maintained by Banaras Hindu University, the

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http://dx.doi.org/10.1016/j.fcr.2013.08.008

Table 1
Mean spot blotch severity (% severity), area under disease progress curve (AUDPC) % days and key morphological traits of the parents used in the crossing programme.

Parents		Mean disease response			
		% severity		AUDPC % days[a]	
Resistant	Other traits	2005–2006	2006–2007	2005–2006	2006–2007
EMBSN-27-4-1	Anthocyanin absent	25.4 ± 4.8	22.6 ± 3.7	464 ± 84	419 ± 89
BCU 455	Dwarf	20.0 ± 3.3	25.4 ± 4.8	390 ± 77	431 ± 97
BCU 570	Erect	25.4 ± 4.8	22.6 ± 3.7	423 ± 73	442 ± 75
HMMBSN-7-47	Anthocyanin present	20.0 ± 3.3	25.4 ± 4.8	385 ± 84	411 ± 88
Susceptible					
RD 2624	Drooping	89.4 ± 5.3	90.4 ± 5.0	2338 ± 243	2176 ± 223
RD 2614	Tall	84.3 ± 4.9	89.0 ± 5.0	2001 ± 195	2206 ± 210
RD 2503	Non-waxy	92.4 ± 5.3	90.4 ± 5.0	2250 ± 220	2131 ± 215
CIHO 3510	Anthocyanin absent	84.3 ± 4.9	89.0 ± 5.0	2095 ± 202	2289 ± 235

[a] (AUDPC/days to maturity) × 100.

Directorate of Wheat Research and the National Bureau of Plant Genetic Resources of Indian Council of Agricultural Research (ICAR). Many of the accessions were developed by either CIMMYT or ICARDA. Both two- and six-rowed cultivars were represented. The material was sown as three replicates in a randomized complete block design at Agricultural Research Farm, Banaras Hindu University, Varanasi, India (25.2° N, 83.0° E) during the first fortnight of December in 2005, 2006 and 2007. Each of the 1662 accessions was drill sown in a paired row plot of 3 m length with 25 cm spacing between the rows and 5 cm between seeds. Two rows of the highly susceptible variety K603 were planted between plots one week prior to sowing the main experiment, and two rows of the same variety were included after each 20 rows of the germplasm set. Standard agronomic practices were followed in each year; while potassium and phosphate were both given at the time of sowing, the nitrogen application was split into three – 30 kg per ha at sowing, 15 kg at 21 days after sowing and 15 kg at 45 days after sowing. Four irrigations were given in each season.

2.2. Disease inoculation and assessment

A highly purified culture of the pathogen (RCBHUBR1857), isolated from naturally infected barley (Chand et al., 2002), was multiplied on sorghum grains and the spores suspended in water (Misra, 1973). The experimental plots were infected by spraying during the evening hours with a spore-water suspension (10^4 spores ml^{-1}) first at the time of tillering (Zadoks growth stage 25, see Zadoks et al., 1974), then at flag leaf emergence (growth stage 37) and finally at anthesis (growth stage 65). The development of disease was evaluated on ten plants per accession using the 0–9 scale described by Saari and Prescott (1975) at growth stages 25, 37, 47, 57, 69 and 77, and spot blotch severity (% area of leaf infected with disease) was also recorded at the same time. Accessions with a mean score of 1–3 were classified as resistant, of 4–5 as moderately resistant, of 6–7 as moderately susceptible and of 8–9 as fully susceptible. AUDPC, based on disease severity, was calculated following Roelfs et al. (1992). The AUDPC values were adjusted for maturity following the suggestion of Reynolds and Neher (1997). Days to maturity, grain yield, thousand grain weight (TGW), and spot blotch severity (%) were also recorded. An entry was considered to have reached maturity when at least 50% of the ears were no longer green. TGW was assessed from grain having a moisture content of 12%.

2.3. Association between resistance and various phenotypic traits

The same set of germplasm was grown over three consecutive seasons in a disease-free environment to characterize each accession for waxiness, anthocyanin pigmentation, plant height and leaf angle. The planting design was identical to that used in the disease nursery, with protection against fungal infection provided by the application of 625 g a.i. per ha propiconazole at growth stages 54 and 69.

2.4. Linkage between resistance and whole plant traits

The F_3 and F_4 generations of four resistant × susceptible crosses (Table 1) were used to test for genetic linkage between resistance and the chosen phenotypic traits. Each set of about 200 F_3 families per cross was advanced to F_4 by selecting a single plant at random (Joshi and Chand, 2002). The F_4 generation was represented by the progeny of a single random F_3 plant (Singh and Rajaram, 1991). The F_3 and F_4 populations of each crosses was raised in both a protected and a spot blotch infected environment. The two parents of each cross were sown at each end of the plot and also after every 20th row.

2.5. Phenotypic scoring

Waxiness of the leaf sheath, leaf blade and spike was scored at growth stage 69. Each accession was considered to be either waxy or non-waxy. Anthocyanin pigmentation reflected the colour of the lower node of the stem at growth stage 59; the accessions were scored as being either anthocyanin present or anthocyanin absent. Plant height was measured at growth stage 77; accessions taller than 95 cm were considered as tall, and those shorter than this as dwarf. Leaf angle was measured shortly after ear emergence (growth stage 51–55), as recommended by Joshi et al. (2002), using a protractor; the accessions were classified as either erect (flag leaf non-drooping, forming an angle of 60–90° with the horizontal), semi-erect (flag leaf non-drooping, angle of 0–60°); semi-drooping (less than half the length of the flag leaf drooping) or drooping (more than half the length of flag leaf drooping).

2.6. Statistical analysis

An analysis of variation was performed to test for the significance of differences in levels of resistance and the plant traits. An unpaired *t* test was used to compare the AUDPCs.

3. Results

3.1. Genetic variation for spot blotch resistance

The analyses of variance for spot blotch AUDPC, time to maturity and TGW all indicated that a significant degree of genotypic variation existed among the accessions (Table 2). Only 5% of the