

BRACHYPODIUM DISTACHYON: WHAT'S IN IT FOR US?

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From the ancient times humanity has been growing cereals, with wheat, corn, and rice currently being able to provide nearly two thirds of the global caloric intake [1]. Fast growing world population rate raises up the issue of human-wise crop production and distribution with the necessity of distinct sustainability. Study on attributes of plant immunity, especially in chosen based on their suitability for research model plants facilitates understanding the mechanisms of plant resistance and improve disease management strategies [2; 3]. A recently novel object for scientific research *Brachypodium distachyon* shares common characteristics of a model plant with its small genome, small physical plant size, a short lifecycle, and less demanding growth requirements similar to *Arabidopsis thaliana*, and unlike the latter being phylogenetically close to important temperate grasses namely wheat, rye, triticale, and barley of tribe *Triticeae* in *Poaceae* family best placed in its own tribe *Brachypodieae* being able to get infected by selected grass rust pathogens, including yellow, leaf and stem known as the “polio of agriculture”, a frequent threat for wheat production in Kazakhstan as well as worldwide, a challenge especially interesting in terms of discoveries in the field of nonhost resistance [4-10].

According to the Dictionary of the Fungi, the *Puccinia* genus contains about 4000 species with spread from Africa to South and Central Asia, the Middle East and Europe leading to a mounting concern at the dangers posed to global food security [11-13]. As mentioned above the major three representatives of rusts are: yellow (stripe), leaf (brown) and stem. “Yellow rust” caused by *Puccinia (P.) striiformis* takes its name from the appearance of yellow-colored stripes produced parallel along the venations of each leaf blade. These yellow stripes are actually characteristic of uredinia that produce yellow colored urediniospores. Not only the bread wheat, but durum wheat, triticale, as well as a few (barley) cultivars play the role of its primary hosts with common barberry plants (the alternate host of wheat stem rust) being a potential alternative host as discovered by accident in 2010 [14]. High humidity and rainfall are favorable conditions for increasing the infection on both leaf blade and leaf sheath, even on spikes when in epidemic form. Symptoms are stunted and weakened plants, shriveled grains, fewer spikes, loss in number of grains per spike and grain weight. Losses can be 50%, but in severe situation 100% is vulnerable. In countries where wheat is grown in winters or at high elevations, yellow rust is a common threat, but not more significant than wheat leaf rust and stem rust, which are continuous threats in all wheat-growing countries. In 1946 Chester provided one of the first detailed histories of published literature on the rusts. The Italians Fontana and Tozzetti independently provided the first unequivocal and detailed reports of wheat stem (black) rust in 1767 with the causal organism named *P. graminis* by Persoon in 1797. An epidemic of stem rust on wheat caused by race TTKSK (e.g. isolate Ug99) is currently spreading across Africa, Asia and the Middle East and is causing major concern due to the large numbers of people dependent on wheat for sustenance. However, this goes even longer back into the history. Excavations in Israel have revealed urediniospores of stem rust that have been dated at about 1300 B.C. Aristotle (384-322 B.C.) writes of rust being produced by the “warm vapors” and mentions the devastation of rust and years when rust epidemics took place. “Stern Robigo, spare the herbage of the cereals, ...withhold we pray thy roughening hand...” was part of the official prayer at a Robigala ceremony as given by Ovid (43 B.C.-17 A.D.) and gives the impression that stem rust was a serious disease in Italy during that time [15-17]. Till 19th century wheat leaf rust is not distinguished from stem rust. However, by 1815 de Candolle had shown that wheat leaf rust was caused by a distinct fungus and described it as *Uredo rubigo-vera*. The pathogen underwent a number of name changes until 1956 when Cummins and Caldwell suggested *P. recondita* (sometimes reconciled as *P. triticina*), which is the generally used nomenclature today for a

generally uniform infection across a crop, which can start as “hot spots” (foci) of infection in paddock from which disease rapidly spreads within and between wheat crops. There are several areas worldwide in which each of the rusts can cause severe losses [18-20]. In other areas, the environment is marginally suited for the diseases (Table 1).

Table 1 – Environmental conditions required for the wheat rusts [21]

Stage	Temperature (⁰ C)			Light	Free water
	Minimum	Optimum	Maximum		
Leaf rust					
Germination	2	20	30	Low	Essential
Germling	5	15-20	30	Low	Essential
Appressorium	-	15-20	-	None	Essential
Penetration	10	20	30	No effect	Essential
Growth	2	25	35	High	None
Sporulation	10	25	35	High	None
Stem rust					
Germination	2	15-24	30	Low	Essential
Germling	-	20	-	Low	Essential
Appressorium	-	16-27	-	None	Essential
Penetration	15	29	35	High	Essential
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None
Stripe rust					
Germination	0	9-13	23	Low	Essential
Germling	-	10-15	-	Low	Essential
Appressorium	-	-	(not formed)	None	Essential
Penetration	2	9-13	23	Low	Essential
Growth	3	12-15	20	High	None
Sporulation	5	12-15	20	High	None

Apparently, even those environmental conditions which are generally regarded as favorable might not in fact be so, what is frequently dependent on their intensity and extent. For instance, rain favors disease by scrubbing spores from the air, depositing them on the plants and increasing the humidity. However, rain can also wash spores from the plant surfaces, and high humidity restricts spore movement. The change in temperature due to rain will influence disease progress [21]. Plants in turn are trying to protect themselves against unwelcomed guests displaying a set of defense responses, including cultivar or accession specific, such as hypersensitive response (rapid localized cell death at the site of infection), increased expression of defense (pathogenesis) – related genes and the oxidative burst, and nonspecific (often nonhost) reactions, operating under less-understood mechanisms, performed against the invading pathogens. Recent advances in molecular biology of plant pathogen interactions suggest that adapted pathogens are able to suppress the basal defence of their host plants, but not in, sometimes related, non-host plant species [22; 23].

Brachypodium distachyon is a host to a rust pathogen species, *P. brachypodii*, which infects a number of *Brachypodium* spp. Phylogenetic studies based upon ribosomal RNA intergenic spacer sequences indicate that *P. brachypodii* is more closely related to *P. striiformis* than other rust species, including *P. graminis* and *P. tritricina*. Resistance to an isolate of *P. brachypodii* was shown to be quantitatively inherited in a cross between *B. distachyon* accessions Bd3-1 and Bd1-1, with three quantitative trait loci identified that collectively explained 39 to 54% of the variance in seedlings and 22% of the variance in older plants [24-28].

Comparative study of molecular genetic and biochemical features of the model of wild cereal *Brachypodium distachyon* with related cereal grains enables us to understand mechanisms of resistance and increase of resistance of wheat plants to both abiotic and biotic factors. A change in the activity of enzymes of metabolism is one of the significant criteria changes the genetic apparatus exposed to mutagens. In this regard, of particular interest is the study of the activity of several key enzymes of nitrogen and energy metabolism in mutant genotypes as compared to the initial variety, which makes it possible to judge the intensity of the metabolism in plant, and more reliably estimate the vitality of these mutant genotypes. The increased interest to the composition of storage proteins in wheat is associated with functional significance of specific proteins in determination of the baking properties. Refinement of genetic control and the identification of new and rare protein subunits, detected in the course of studying the collection and breeding material is necessary for a reliable assessment of samples, as well as for the expansion of the genetic basis of cultivars created by examining the value of genotypes with specific variants of alleles and their inclusion in the selection process.

In our recent publication we have shown that xanthine dehydrogenase activity as an indicator of the degree of oxidative stress and of resistance on the test plants under biotic stress showed that its activity in the leaves of Kazakhstanskaya early and Kazakhstanskaya 19, which according to the results of evaluations by the laboratory of plant immunity and protection of Kazakh Research Institute of Agriculture and Crop Production and Research Institute for Biological Safety showed resistance to brown (14%) and yellow rust, after infection with pathogen increased slightly (at 10 and 5% in comparison to control), whereas in *Brachypodium* infected as well by the Kazakhstani strain of *Puccinia recondita* it decreased by 36% in comparison to control. Based on these data it can be concluded that the inhibition of xanthine dehydrogenase activity in *Brachypodium distachyon* may be associated with the increased plant resistance to pathogen action. In accordance with our findings we can conclude that this enzyme plays an important role in the process of plant adaptation to the fungus *Puccinia recondita* [29].

We have also studied the response of *Brachypodium* plants to infection with rust spores in the spectrum of storage proteins fractionated in alkaline and acidic systems. For the first one the range of storage proteins has slow-moving high-molecular subunits, similar to glutenin in cereals – wheat, barley, corn. In acidic system components in the middle part of the gel, apparently, are not prolamins; these proteins are not detected or are present in trace amounts. Fast moving proteins related to wheat albumin-globulin fraction are observed as well. According to Larre C. et al. (2010) [30], who studied the protein composition of *Brachypodium* grain, salt-soluble proteins as well as salt-insoluble proteins separated by two-dimensional gel electrophoresis were revealed as 284 and 120 spots, respectively. Proteins from the major spots were sequenced by mass spectrometry and identified by searching against a *Brachypodium* putative protein database. The authors found prolamins and globulins, no albumins were found. Globulins were represented mainly by the 11S type and their solubility properties corresponded to the glutelin found in rice. Microscopic examination of endosperm cells revealed scarce small-size starch granules surrounded by protein bodies containing 11S globulins. According to the authors, the presence of protein bodies containing glutelins makes *Brachypodium distachyon* closer to rice or oat than to wheat endosperm. In another study by Laudencia-Chinguanco D.L. and Vensel W.H. (2008) seven major protein groups, six of which have been identified as globulins, were found using sodium dodecyl sulfate polyacrylamide gel electrophoresis and mass spectrometry. A subset of the major storage proteins extracted from three hexaploid accessions, Bd4, Bd14 and Bd17 has also been identified as globulins. Several clones of *Brachypodium*, encoding globulins were completely sequenced. Two types of globulin genes were identified, Bd.glo1 and Bd.glo2, which are similar to maize 7S and oat 12S globulins, respectively. The derived polypeptide sequences of the globulins contain a typical signal peptide sequence in their polypeptide N-termini and two cupin domains. Bd.glo1 is encoded by a single copy gene, whereas, Bd.glo2 belongs to a gene family [31]. The reduction of the intensity of expression in the spectrum of storage proteins of *Brachypodium distachyon* is not observed in the experimental

samples in comparison to control [29].

To conclude, it should be emphasized that beside the importance of *Brachypodium* as a model plant, including but not limited to revealing the mechanisms of rust nonhost resistance which will bring us a much better view of the dynamics of plant disease resistance, research in this species can be useful only if it is done side-by-side and complementarily to studies on related crop species.

Citations

1. Tilman D., Balzer C., Hill J., Befort B.L. Global food demand and the sustainable intensification of agriculture // *Proc. Natl. Acad. Sci. USA*, 2011. Vol.108, pp. 20260–20264.
2. Figueroa M., Castell-Miller C.V., Li F., Hulbert S.H., Bradeen J.M. Pushing the boundaries of resistance: insights from *Brachypodium*-rust interactions // *Front Plant Sci.*, 2015. Vol. 6, No. 558. 11 pp.
3. Müller B., Grossniklaus U. Model organisms – a historical perspective // *J Proteomics*, 2010. Vol. 73, pp. 2054–2063.
4. Watson L., M.J. Dallwitz R. The grass genera of the world. Oxon: Wallingford, CAB International, 1992. 1038 p.
5. Catalfin P., Olmstead R.G. Phylogenetic reconstruction of the genus *Brachypodium* P. Beauv. (Poaceae) from combined sequences of chloroplast *ndhF* gene and nuclear ITS // *Plant Syst. Evol.*, 2000. Vol. 220, pp. 1-19.
6. Shi Y., Draper J., Stace C.A. Ribosomal DNA variation and its phylogenetic implication in the genus *Brachypodium* (Poaceae) // *Plant Syst. Evol.*, 1993. Vol. 188, pp. 125-138.
7. Meineke T., Manisseri C., Voigt C.A. Phylogeny in defining model plants for lignocellulosic ethanol production: a comparative study of *Brachypodium distachyon*, wheat, maize, and *Miscanthus x giganteus* leaf and stem biomass // *PLOS One*, 2014. Vol. 9, No. 8, e103580, pp. 1-14.
8. Kazakhstan wheat production review <http://www.world-grain.com/Departments/Country-Focus/Country-Focus-Home/Kazakhstan-2016.aspx?cck=1> (Post from May 4, 2016).
9. Ayliffe M., Singh D., Park R., Moscou M., Pryor T. Infection of *Brachypodium distachyon* with selected grass rust pathogens // *MPMI* 2013. Vol. 26, No. 8, pp. 946–957.
10. Wheat rust: The fungal disease that threatens to destroy the world crop. <http://www.independent.co.uk/news/uk/home-news/wheat-rust-the-fungal-disease-that-threatens-to-destroy-the-world-crop-9271485.html> (Post from April 19, 2014).
11. Kirk P.M., Cannon P.F., Stalpers J.A., Minter D.W. Dictionary of the Fungi. 10th ed., CSIRO, 2011. 784 p.
12. Joining forces to defeat wheat disease: UK-South Africa collaboration to tackle global threat Feb.2012: <http://www.foodsecurity.ac.uk/research/current/defeating-wheat-disease.html>.
13. Rust never sleeps post from March 03, 2011 by Ronnie Coffman at: <http://www.impatientoptimists.org>.
14. Jin Y., Szabo L.J., Carson M. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host // *Phytopathology* 2010. Vol. 100, No. 5, pp. 432-435.
15. Wheat CAP by J. Sherman: <http://maswheat.ucdavis.edu/education/PDF/facts/rustfacts.pdf>
16. <http://www.apsnet.org/edcenter/intropp/lessons/fungi/Basidiomycetes/Pages/StemRust.aspx>
17. Roelfs A.P., Singh R.P., Saari E.P. Rust diseases of wheat: concepts and methods of disease management. Mexico, D.F.: CIMMYT, 1992. 81 p.
18. Government of Western Australia Department of Agriculture and Food <http://www.agric.wa.gov.au/mycrop/diagnosing-leaf-rust-wheat>. Last updated May 3, 2016.
19. Abbasi M., Ershad D., Hedjaroude G.A. Taxonomy of *Puccinia recondita* s. lat. causing brown rust on grasses // *Iran J Plant Path*, 2005. Vol. 41, No. 4. pp. 631–662.

20. Saari E.E., Prescott J.M. World distribution in relation to economic losses. In A.P. Roelfs & W.R. Bushnell, eds. *The cereal rusts*, Vol. 2, Diseases, distribution, epidemiology, and control, pp. 259-298. Orlando, FL, USA, Academic Press, 1985.
21. The wheat rusts by Singh R.P., Huerta-Espino J., Roelfs A.P. at: <http://www.fao.org>
22. Schulze-Lefert P., Panstruga R. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation // *Trends in Plant Science*, 2011. Vol. 16, No. 3, pp. 117-125.
23. Battepati Uma, T. Swaroopa Rani, Appa Rao Podile. Warriors at the gate that never sleep: Non-host resistance in plants // *J Plant Physiol.*, 2011. Vol.168, No.18, pp. 2141-2152.
24. Fitzgerald T.L., Powell J.J., Schneebeli K. et al. *Brachypodium* as an emerging model for cereal-pathogen interactions // *Annals of Botany*, 2015. 115. 717-731.
25. Barbieri M., Marcel T.C., Niks R.E. Host status of false brome grass to the leaf rust fungus *Puccinia brachypodii* and the stripe rust fungus *P. striiformis* // *Plant Disease*, 2011. Vol. 95, pp. 1339–1345.
26. Zambino P.J., Szabo L.J. Phylogenetic relationship of selected cereal rusts based on rRNA sequence analysis // *Mycologia*, 1993. Vol. 85, pp. 401–414.
27. Barbieri M., Marcel T.C., Niks R.E. et al. QTLs for resistance to the false brome rust *Puccinia brachypodii* in the model grass *Brachypodium distachyon* L. // *Genome*, 2012. Vol.55, pp. 152–163.
28. Zhang X., Han D., Zeng Q. Fine mapping of wheat stripe rust resistance gene Yr26 based on collinearity of wheat with *Brachypodium distachyon* and rice // *PLoS ONE*, e578852013.
29. Omirbekova N.Zh., Zhussupova A.I., Askanbayeva B.N. et al. Study of storage proteins in endosperm and antioxidant enzyme activity of soft wheat and *Brachypodium distachyon* infected by *Puccinia recondita* // *SGEM*, 2016. Vol. II, pp. 767-774.
30. Larre C., Penninck S. et al. *Brachypodium distachyon* grain: identification and subcellular localization of storage proteins // *J Exper. Botany*, 2010. Vol. 61, No. 6, pp 1771-1783.
31. Laudencia-Chingcuanco D.L., Vensel W.H. Globulins are the main seed storage proteins in *Brachypodium distachyon* // *Theor. Appl. Gen.*, 2008. Vol. 117, pp. 555563.