Acid soil tolerance of cereals. Significance, present situation and prospects

L. BONA, L. CSEUZ and A. MESTERHAZY

Introduction

Acid soils (pHd“5.5) significantly limit crop production all over the world and the acidification is a harmful progress in the last decades. In the seventies, a report said that problem acid soils are near 3 billion hectares in the globe (Dudal, 1976). Twenty years later, the survey of von UEXKÜLL and MUTERT (1995) stated that 1/3 of the earth surface soil is acidic (cca 4 billion ha) and from this, 178 million ha-s are agricultural soils. A more recent announcement of Kochian et al. (2005) declares that approximately 50 % of the world’s potentially arable soils are acidic.

The primary limitations on acid soils are toxic levels of aluminum (Al) and manganese (Mn), as well as suboptimal levels of phosphorous (P), calcium (Ca) and other beneficial elements. In fact, much of the damage to plant production on acid soils is due to Al toxicity. The most striking effect of Al in acid soils is stunting the root system. Toxic aluminum (Al) cations solubilized by the acidity rapidly inhibit root growth and limit subsequent uptake of water and nutrients (BONA et al. 1991, FOY 1983, BALIGAR and FAGERIA 2001). Thus, most of the research on plant-soil interaction at low pH has focused on the root and root growth problems.

While liming is a common and efficient practice to ameliorate topsoil acidity, in some cases it can not be real solution. Sometimes the distance from the lorry is the reason that it is highly expensive solution. Growing Al-tolerant crops may provide an additional strategy to fight the subsurface acidity problem. The present study focuses on some theoretical approaches, highlights trends and efforts, and offers practical methods to improve the acid soil tolerance of small grain cereal species.

Physiological and genetic aspects

Physiological and genetic studies in the nineties cleared up many aspects of the acid soil tolerance of cereals. Because the primary response to the stress occurs in roots, this is the most researched object. Many studies revealed that reduction in root elongation may occur as short as 2 h time period (OWNBY and POPHAM, 1989, ZSOLDOS et al. 1998). In sorghum, contents of the main organic acids found in the roots and leaves increased with plant exposure to Al and were always higher in the aluminum (Al)-tolerant cultivar. Malic and taconitic acids were the most abundant and showed the highest absolute changes in the presence of Al in both cultivars, especially the Al-tolerant cultivar. Aluminum also changed the activities of most of the enzymes related to organic metabolism studied (GONCALVES et al. 2005).

The enhanced Al tolerance exhibited by some wheat cultivars is associated with the Al-dependent efflux of malate from root apices. Malate forms a stable complex with Al that is harmless to plants and, therefore, this efflux of malate forms the basis of a hypothesis to explain Al tolerance in wheat (SASAKI et al. 2004).

US-researchers recently reported that evidence for the organic acid (OA) secretion hypothesis of resistance is substantial, but the mode of action remains unknown because the OA secretion appears to be too small to reduce adequately the activity of Al3+ at the root surface. According to their computations, Al3+ activity is insufficiently reduced at the surface of the root tips to account for the Al resistance of Triticum aestivum L. cv. Atlas 66, a malate-secreting wheat. Experimental treatments to decrease the thickness of the unstirred layer (increased aeration and removal of root-tip mucilage) failed to enhance sensitivity to Al3+. On the basis of additional modelling, the observed spatial distribution of Al in roots, and the anatomical responses to Al, it is proposed that the epidermis is an essential component of the diffusion pathway for both OA and Al. They suggest that Al3+ in the cortex must be reduced to small concentrations in order substantially to alleviate the inhibition of root elongation and so that the outer surface of the epidermis can tolerate relatively large concentrations of Al3+. If OA secretion is required for reducing Al3+ mainly beneath the root surface, rather than in the rhizosphere, then the metabolic cost to plants will be greatly reduced (KINRAIDE et al. 2005).

Production of root mucilage is an essential path of tolerance. Horst et al (1982) demonstrated that removal of root cap mucialage caused an increase in Al uptake with significant phytotoxicity. HENDERSON and OWNBY (1991) reported a strong association between the volume of root mucilage and root production in Al stress; however the protection-mechanism by mucilage is not clear yet. Recent studies indicated that callose and lignin formation can be used as interspecific indicators of Al sensitivity (TAHARA et al. 2005).

Japanese scientists investigated the rapid modification of plasma membrane and changes in mineral nutrients in root-tip cells of Al-tolerant rice and Al-sensitive barley following short-term exposure to Al (20 muM Al, 1 h). The plasma membrane of the barley cells was significantly permeabilized when re-elongated in an Al-free Ca solution following a 1-h pretreatment with Al, while that of rice cells was not affected at all. Al was localized primarily to the epidermis and outer cortex cells in both species, and was much more abundant in barley than in rice. Al increased and decreased remar-

Autoren: Dr. Lajos BONA, Dr. László CSEUZ and Prof. Dr. Akos MESTERHAZY, Cereal Research NPC, P.O.Box 391, H-6701 SZEGED, lajos.bona@gk-szeged.hu

kably the intracellular K concentration in whole root-tip cells of rice and barley, respectively. In barley, the decrease in the concentration of Ca coincided with the accumulation of Al. Conversely, the intracellular concentration of P in the surface layers of root-tip cells increased with the accumulation of Al. The distribution and concentration of Ca and P in rice did not change after 1-h treatment with Al. These results also suggest that the rapid modification of the plasma membrane of root-tip cells induced by Al affects the nutritional homeostasis in the cells (ISHIKAWA et al. 2003).

After a twenty year of research in physiology and genetics, basically, two main classes of tolerance mechanisms have been proposed to account for Al tolerance in plant roots. The first is that allow the plant to tolerate Al in the symplasm it is called the real Al tolerance. Those that exclude Al from the root apex are called Al exclusion (or Al resistance). Besides, emerging area of the P efficiency, which involves the genetically based ability of some crop genotypes to tolerate P deficiency stress on acid soils. (KOCHIAN et al. 2004).

Molecular-assisted biotechnology

These are interesting times for this field because researchers are on the border of identifying some of the genes that confer tolerance/resistance in crop plants; these discoveries will open up new pathways in molecular/physiological inquiry that should greatly advance the theoretical understanding of mechanisms and shall effect on practical breeding as well.

Recent work of Australian and Japanese researchers highlighted the ALMT1 gene of wheat (Triticum aestivum) encoding a malate transporter that is associated with malate efflux and Al tolerance. They generated transgenic barley (Hordeum vulgare) plants expressing ALMT1 and assessed their ability to exude malate and withstand Al stress. ALMT1 expression in barley conferred an Al-activated efflux of malate with properties similar to those of Al-tolerant wheat. The transgenic barley showed a high level of Al tolerance when grown in both hydroponic culture and on acid soils. These findings provide additional evidence that ALMT1 is a major Al-tolerance gene and demonstrate its ability to confer effective tolerance to acid soils through a transgenic approach in an important crop species (DELHAIZE et al. 2004, SASAKI et al. 2004).

Testing in soil: field trials and short screening method

Basically, two main testing media exists soil, and solution cultures.

Both have advantages and disadvantages. Testing cereals in soil is more realistic - close to the practice- but less accurate. Testing the plant response in solution culture is more precise elucidation.

Field trials

Australian researchers compared the growth, water use and yield of two near-isogenic wheat genotypes differing only in Al tolerance in response to subsurface acidity in the field (TANG et al. 2002). The trial was conducted on a sandy soil in the low-rainfall region of Western Australia, and received 130 mm rainfall during the growing season. The soil had pH 4.4 and extractable Al 5.2 mg kg^{-1}. Seven irrigation treatments and two wheat genotypes (Al-tolerant ET8 and Al-sensitive ES8) were applied. The water treatments were natural rain, weekly, fortnightly and monthly irrigation. Tolerant genotype ET8 produced more shoot biomass than ES8 from 34 days after sowing. At maturity, ET8 produced 51 % higher yield than ES8 under natural rain. Under irrigation, ET8 produced up to 26 % higher yield than ES8. While both genotypes had similar root length density in the topsoil, root length density in the 10 - 40 cm layer was 20 - 50 % higher in ET8 than in ES8. Soil water was depleted faster under ET8 than under ES8 in soil layers between 10 and 110 cm. Under irrigation, the decrease in water content was not evident below 70 cm for ES8 and below 110 cm for ET8, indicating that ET8 roots grew deeper than those of ES8. The results suggest that the higher yield of Al-tolerant wheat than Al-sensitive wheat grown with subsurface acidity results from the greater root proliferation and hence water use in the subsurface layers.

Quick root bioassay

The bioassay method was developed in the early nineties in the USDA, Appalachian Research Laboratory, Beaver, West Virginia where I served as a visiting researcher (BONA et al. 1991). There are tough acidic lands in the region and testing plants for sustainable production is an important issue in farming and research. The method is based on the root production, actually the abilities of the tested entries for rooting in stress versus non-stress position. Limed and unlimed version of the target soil is used in the test. We used Porters soil (coarse-loamy, mixed, mesic Umbric Dystrochepts) for cereal testing. The limed (+L) version consisted of an addition of dolomitic lime at a rate of 4 g/kg soil to reduce Al toxicity with pH 5,2 - while pH of the unlimed version of the soil was 4.2. Seeds were germinated in Petri dishes. Twelve uniform, healthy seeds were selected from each entry and planted at a rate with four seedlings per 200 mL plastic cup. Each cup contained 200 g of soil packed to a bulk density to 33kPs moisture tension. Three replications (three cups) of both the imed and unlimed treatments of the soil were arranged in a randomized complete block design. The cups were placed on trays containing moist paper towels and covered with a plastic dome providing a humid atmosphere to maintain the desired moisture level. Soft, uniform spraying of the soil is required to avoid drying of the soil surface. Plants were grown for 3 days in a growth chamber set at 80 % relative humidity and 20 ^{o}C with 12 h per day light illumination. The longest root of each seedling measured at harvest and average longest root length for each replication, entry and species are calculated (ALRL). In early tests (WRIGHT et al. 1989), length of the longest root (LR) has been shown to be correlated with total root system length in wheat thus, LR is a characteristic and sufficient trait for this aim. Acid soil tolerance index (Ti) was calculated for each entry by dividing the ALRL (-L) by the ALRL (+L). Wheat cultivars with known tolerance levels should be used as standards in the tests. Successful adaptation of the method was mentioned by some Canadian and American scientists when determining the value of the tolerance genes, prior to future on-farm validation (BRIGGS and TAYLOR 1993). The bioassay method can be useful in tough acid lands when screening and selecting within segregating populations. Seedlings are still viable and transferable at the end of the test and that is also a sound breeding point.

Screening in nutrient solution

In spite of the tremendous advantages of the soil tests, the most common screening medium for Al and Mn tolerance is solution culture providing easy access to root systems, tight control over the pH and other chemical compositions, and nondestructive measure of the plants. Nutrient solution experiments are designed around two main objectives. The applied *concentrations* and *duration times* of stresses are varied widely. Long (3-6 weeks) exposure to the stress requires low concentrations of Al, while quick (18-24 hr) tests much higher - about 0,4-0,9 mM Al. POLLE et al. (1978), and later CARVER et al. (1991) recommended a qualitative scale to rate wheat genotypes. They called the test hematoxylin staining method - which is an extremely powerful screen for Al tolerance in small grain species. Its biological basis is derived from complexes believed to form between hematoxylin and $AlPO_4$ precipitated in intercellular spaces (CARVER and OWNBY 1995). Brett CARVER was one of the first who suggested that the in most of the case, a single genetic model does not consistently explain the inheritance of Al tolerance, rather, in many crossings, it is quantitative in nature. Later, we proposed a scale to quantify the tolerance level of various wheat genotypes (BONA and CARVER 1998). Our protocol has been to absorb seeds for one d, transfer the seeds to trays suspended above aerated deionized water for two days and to nutrient solution for two additional days. Al is added to the solution in the fifth day (for 24 hours). Seedling roots sooked in hematoxylin are stained along the vertical axis with increased intensity in susceptible genotypes. Particularly the meristamic region, the root tips are stain deeply. Tolerant genotypes have got nonstained patterns. At each level (0.18, 0.36, and 0.72 mM Al concentration) approximately 5 mm of the primary root tip of an entry is scored as completely (C), partially (P), or not (N) stained. Based on the staining patterns, a given genotype can be classified to various tolerance groups (susceptible, intermediate, tolerant, and subclasses). A strong positive correlation was found when compared the hematoxylin method and soil bioassay. Tolerant wheat entries showed reasonably high root tolerance indexes in the soil bioassay study. The repeatability and simplicity of the hematoxylin method makes it highly ranked in breeding and research.

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