

Characterization of Newly Developed Wheat/Barley Introgression Lines in Respect of Aluminium Tolerance

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ABSTRACT

The Al tolerance of newly developed wheat/barley disomic addition, substitution and translocation lines carry chromosomes of three different barley cultivars was evaluated by comparing the root growth in solution containing 75 μ M AlCl₃ at pH 4.0 to that of known Al-tolerant and sensitive wheat genotypes. The wheat Asakaze komugi, barley Manas cultivars and their hybrid derivatives were found to have high levels of Al tolerance. The wheat line Mv9kr1, barley cultivar Igri and progenies of the hybrids were sensitive to Al. In most cases, the Al tolerance of the wheat/barley introgression lines derived from Al-sensitive wheat Mv9kr1 and barley Betzes with moderate Al tolerance was similar to that of the wheat parents, but the 2DS.2DL-1HS translocation line of Mv9kr1/Betzes exhibited more intensive root growth, while accumulating less Al than the parental lines. This indicates that either the lack of the distal part of chromosome 2DL or the presence of the distal part of 1HS improved the Al tolerance level.

Keywords: Aluminium Tolerance; Barley; Introgression Line; Wheat

1. Introduction

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are important cereals worldwide. The sexual hybridization of these species makes it possible to transfer agronomically useful genes into wheat, such as genes responsible for earliness, drought tolerance and nutrition quality. The first successful hybridization between wheat and barley was reported by Kruse [1] and not much later a set of wheat/barley addition lines was produced [2]. The addition lines were produced from Chinese Spring wheat and Betzes barley cultivars which have high crossability in intergeneric crosses but are unsatisfactory from the agronomic point of view. Very few new hybrid combinations have been reported from wheat \times barley crosses since then and in most cases no backcross progenies were developed [3,4].

Recently, new wheat \times barley hybrids (wheat Mv9kr1 \times barley Igri; wheat Mv9kr1 \times barley Betzes; wheat Asakaze komugi \times barley Manas) have been produced by Molnár-Láng *et al.* [5-7]. Partial sets of addition, substitution and translocation lines were then developed from these hybrids and identified by molecular genetic and cytogenetic methods [6-8]. The agronomic characters of

the new wheat/barley hybrid derivatives are not known thus experiments were carried out to analyse the tolerance of these lines to various environmental stress factors [9,10]. The tolerance level of the wheat/barley addition and translocation lines and their parental genotypes to Al toxicity has not been yet investigated, with the exception of the barley Igri which has low to moderate Al tolerance [11].

Aluminum is the most abundant metal in the earth's crust and becomes toxic to plants when solubilised into the soil solution at acidic pH values (below pH 5.0). The main symptom of the Al toxicity is the inhibition of the root growth, resulting in the poor uptake of water and nutrients from the soil. This ultimately leads to a significant reduction in crop yields on acid soil. Since acid soil occurs on about 49% of the arable lands of the world, Al stress represents one of the important constraints for agricultural production worldwide [12]. In cereals, aluminum toxicity causes a yield loss of at least 30% - 40%. Tolerance to Al or acidic soils differs greatly among cereal species. Rye is considered to be the most Al-tolerant, followed by triticale and wheat, while barley is one of the most sensitive to Al toxicity. However, wide genetic variation in Al tolerance has been reported in both wheat and barley [13-15].

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The genetics and chromosome localization of genes responsible for Al tolerance have been extensively studied in cereal crops, including wheat and barley. The members of the ALMT (Al-activated malate transporter) and MATE (multidrug and toxic compound extrusion) gene families were found to have the most pronounced effect on the expression of aluminium tolerance in these species [13,16]. The genes of these families were identified on chromosomes 4DL (*ALMT1*) and 4BL (*TaMATE*) in wheat and on 4HL (*HvAACT1* or *HvMATE*) in barley [17,18]. They encode transport proteins responsible for malate and citrate release. Nevertheless, several minor QTLs controlling Al tolerance were also identified on chromosomes 2A, 5A, 3B and 5D in wheat and 2H, 3H, 5H and 6H in barley, indicating the multigenic trait of Al tolerance [19-21]. In addition, crosses between different genotypes or species have demonstrated that the presence of alien chromosomes may affect the Al tolerance of introgression lines. The expression of alien genes controlling Al tolerance may be restricted in the wheat background. Several suppressor and activator genes were found on chromosome arms 4A, 5A, 7B and 3D and on 2D and 7D, respectively [22,23].

The aim of the present work was to evaluate the Al tolerance of recently developed wheat/barley disomic addition, substitution and translocation lines and of their parental wheat and barley genotypes. The Al tolerance of the plants was determined by monitoring the root growth in a solution containing Al at pH 4.0 and by the root regrowth test after haematoxylin staining. The results were compared to those of known Al-tolerant and sensitive wheat genotypes.

2. Materials and Methods

2.1. Plant Materials

The plant materials used in this study included nine wheat/barley introgression lines, their parental wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) genotypes and three wheat genotypes with known levels of Al tolerance, as controls.

The wheat/barley introgression lines were the following: the disomic addition lines 2H, 3H and 4H of Mv9kr1/Igri [6] and 4H, 6H and 7H of Asakaze komugi/Manas [8], the translocation lines 7DL.7DS-5HS of Mv9 kr1/Igri and 2DS.2DL-1HS and 3HS.3BL of Mv9kr1/Betzes [24] and the substitution line 4H (4D) of Mv9kr1/Betzes [7]. The parental genotypes were: Mv9kr1 (winter wheat line, [25], Asakaze komugi (Japanese, facultative wheat cultivar), Igri (German, two-rowed winter barley cultivar with low to moderate Al tolerance), Manas (Ukrainian, six-rowed winter barley cultivar) and Betzes (North American, two-rowed spring barley cultivar). The wheat geno-

types Chinese Spring (Chinese, moderately Al-tolerant spring wheat cultivar), Atlas 66 (US, Al-tolerant winter wheat cultivar) and Scout 66 (US, Al-sensitive winter wheat cultivar) were used as known Al tolerance controls.

2.2. Determination of Al Tolerance

The Al tolerance of the plants was determined by monitoring the root growth (root length, fresh weight) in nutrient solution containing 75 μM AlCl_3 (pH 4.0) and by root regrowth after haematoxylin staining.

The seeds were surface-sterilized in 10% sodium hypochlorite for 15 min, rinsed twice in distilled water and germinated on moist filter paper in Petri dishes for 2 days at room temperature. Thirty seedlings with similar root length were then grown in nutrient solution (15 plants/1L pot) consisting of 6 mM KCl, 4 mM $\text{Ca}(\text{NO}_3)_2$, 2.5 mM MgSO_4 , 0.5 mM NH_4NO_3 and 75 μM AlCl_3 at pH 4.0. The AlCl_3 concentration was chosen according to pre-experiments. The solutions were renewed every two days. In control experiments the pH was adjusted to 5.5 and 4.0 without Al. The plants were grown for 11 days in a phytotron growth chamber (PGR15, Conviron) under a 16 h photoperiod ($120 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$) at 22°C/20°C day/night with 80% relative humidity.

The haematoxylin test was carried out on 4-day-old seedlings. Fifteen seedlings of each cultivar and line were placed in a solution containing 150 μM AlCl_3 at pH 4.0 for 36 h and then stained with 0.2% haematoxylin. The root elongation was measured after 48h incubation in Al-free solution at pH 4.0.

For the numerical characterization of the Al tolerance of introgression lines and parental genotypes, root tolerance indexes (RTI) were calculated according to [14]. RTI1, representing the tolerance to acid soils, was calculated as the ratio of net root length at pH 4.0 and at pH 5.5. RTI2 values, showing the Al tolerance at pH 4.0, were determined as the ratio of net root length at pH 4.0 with and without Al. The net root length was determined as the average root length after culture minus the average root length before culture in the hydroponic solution.

In addition, the Al content of the roots in the translocation line 2DS.2DL-1HS of Mv9kr1/Betzes) and in the parental cultivars was detected by morin staining according to the method described by [26]. Morin is a metal indicator with high specificity to Al. After blue light illumination, the green fluorescence of the Al-morin complex was detected with Olympus BX 51 fluorescence microscope fitted with a Camedia digital camera (Olympus Optical Co Ltd., Tokyo, Japan).

All the experiments were repeated three times and data presented in the figures and tables are the means \pm SD of three independent experiments.

3. Results

In the present study, the Al tolerance of several wheat/barley disomic addition, substitution and translocation lines and that of the parental genotypes was studied under hydroponic conditions and compared to that of wheat genotypes (Atlas 66, Chinese Spring and Scout 66) with known levels of tolerance.

The mean root lengths of 11-day-old seedlings of wheat and barley genotypes and wheat/barley introgression lines were 11.95 ± 1.63 and 11.2 ± 1.77 cm at pH 5.5 and pH 4.0, respectively without Al treatment. Two parental genotypes exhibited substantial differences from the mean: the root growth of wheat line Mv9kr1 was greater and that of barley Igri less intensive than that of the other genotypes (**Figure 1(a)**). A high growth rate was also observed in the introgression lines originating from the wheat parent Mv9kr1. In addition, more vigorous root growth was observed in the 4H disomic addition lines of Mv9kr1/Igri and 4H of Asakaze komugi/Manas than that in the other two additions from the same cultivar combinations (2H, 3H of Mv9kr1/Igri and 6H and 7H of Asakaze komugi/Manas). Similar result was observed for the

7DL.7DS-5HS translocation line of Mv9kr1/Igri as compared to the parental lines (**Figure 1(a)**). Intensive root growth was also manifested in terms of root mass production (determined as root fresh weight per plant) in wheat genotypes Atlas 66 and Mv9kr1, and in the above-mentioned introgression lines; however, the differences were not so pronounced (**Figure 1(b)**).

Decreasing the pH of the culture medium from pH 5.5 to 4.0 resulted in a slight reduction in root growth in all plants. By contrast, Al stress significantly reduced the root growth of all the wheat and barley genotypes and of their hybrid derivatives (**Figure 1**). As expected, among the wheat genotypes, the reduction in root growth was least pronounced for the Al-tolerant Atlas 66 and greatest for the Al-sensitive Scout 66. The root growth rate was greater in the wheat Asakaze komugi and smaller in the Mv9kr1 wheat line than in the moderately Al-tolerant wheat Chinese Spring (**Figure 1**). In the same Al treatment, the barley Igri had the lowest root length and smallest root weight. Barley cultivars Betzes and Manas exhibited only a slight decrease in root growth under Al stress, showing their tolerance to Al (**Figure 1**).

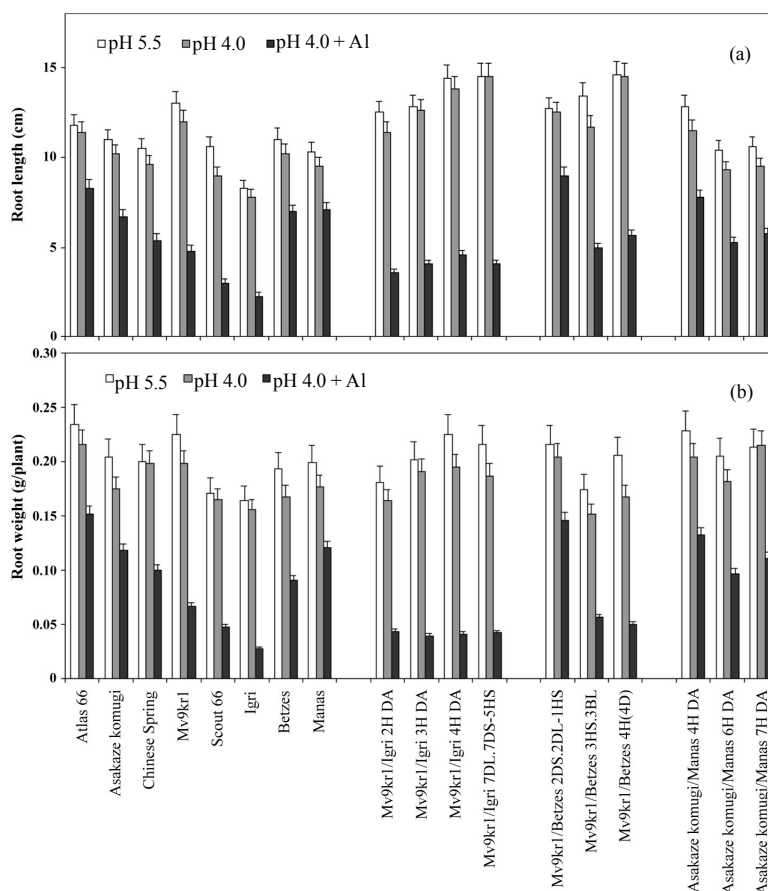


Figure 1. Net root length (a) and root weight (b) of 11-day-old wheat and barley genotypes and wheat/barley introgression lines grown in nutrient solutions at pH 5.5 and 4.0 with and without $75 \mu\text{M AlCl}_3$. The results are means \pm SD of three independent experiments.

The root growth of wheat/barley introgression lines in solution containing Al was primarily determined by the root growth of the wheat parent used in the cross. When the plants were grown in solution containing Al, the root length and weight of the 2H, 3H and 4H disomic addition lines of Mv9kr1/Igri were very small (**Figure 1**). The root growth was also similar in the 3HS.3BL centric fusion and the 4H (4D) substitution lines to that of the parental wheat Mv9kr1. In contrast, the 2DS.2DL-1HS translocation line originating from wheat Mv9kr1 and barley Betzes exhibited higher root growth in solution containing Al than was observed in their parents. The root growth of the 4H disomic addition line of Asakaze komugi/Manas was as high as that found for the wheat Asakaze komugi and barley Manas (**Figure 1**). In the case of the 6H and 7H disomic addition lines of Asakaze komugi/Manas a slight reduction (significant at $p \leq 0.05$) in root growth was detected as compared to the parent Manas (**Figure 1**).

When the Al tolerance of the genotypes was estimated by root regrowth after haematoxylin staining, based on the ability of the seedlings to continue root growth after a short treatment with a high Al concentration, the root regrowth ability was highest in the wheat Atlas 66, high in the barley Manas, medium in the wheats Asakaze komugi and Chinese Spring and the barley Betzes, and low

in the wheat Mv9kr1 (**Figure 2**). The regeneration potential or root regrowth ability after strong Al treatment was practically zero in the wheat Scout 66 and barley Igri genotypes (**Figure 2**).

The root regrowth values of introgression lines originating from wheat Mv9kr1 and barley Igri was intermediate, being slightly lower than that of wheat Mv9kr1 and higher than in barley Igri. However, the differences were not significant. The root regrowth ability in the 3HS.3BL translocation and 4H (4D) substitution lines of Mv9kr1/Betzes was similar to that of the wheat parent Mv9kr1, but in the case of the 2DS.2DL-1HS translocation line of Mv9kr1/Betzes it was comparable to that of the barley Betzes. The root regrowth ability in the 4H, 6H and 7H disomic addition lines of Asakaze komugi/Manas was as high as that recorded for the wheat parent Asakaze komugi (**Figure 2**).

In order to evaluate differences in the response of genotypes and hybrid derivatives to acid pH and Al stress independently of the genetic variation in root growth, root tolerance indexes were calculated (RTI1 and RTI2, respectively) (**Figure 3**). The RTI1 values of the cultivars and the introgression lines were relatively high, and varied over a narrow range from 0.849 to 1.01, indicating that low pH in the culture medium without Al had little effect on root growth (**Figure 1(a)**). In contrast, RTI2

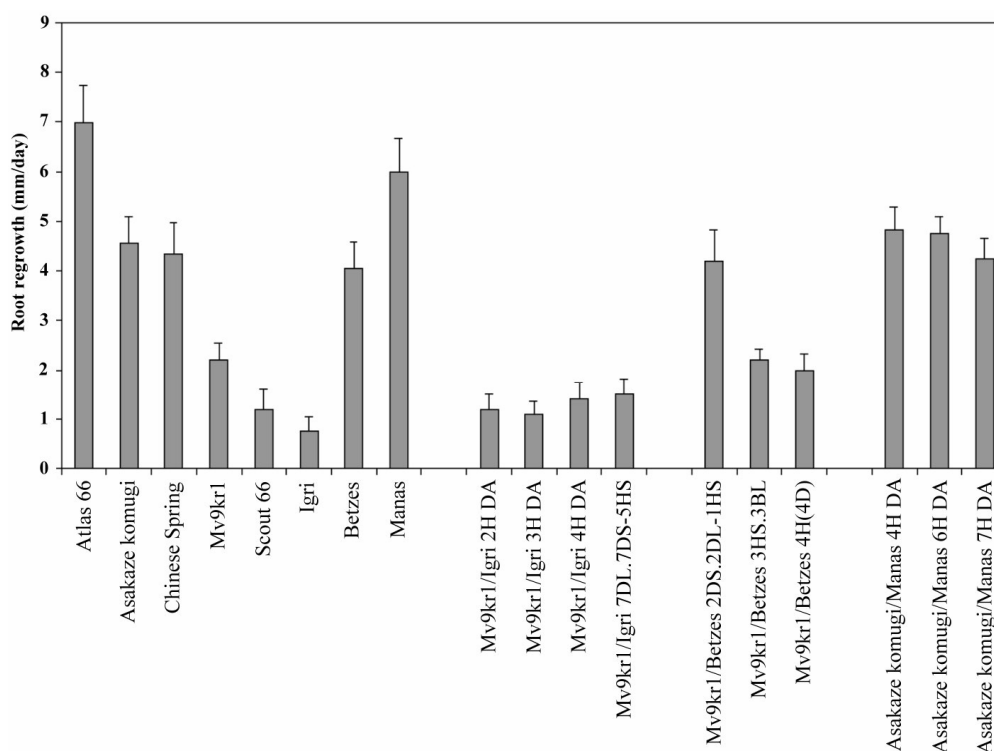


Figure 2. Results of haematoxylin test carried out on 4-day-old seedlings of wheat and barley genotypes and wheat/barley introgression lines. The test is based on the ability of seedlings to continue the root growth after a short (36 h) treatment with high concentration (150 μ M) of Al followed by haematoxylin staining. Data are means \pm SD of three independent experiments.

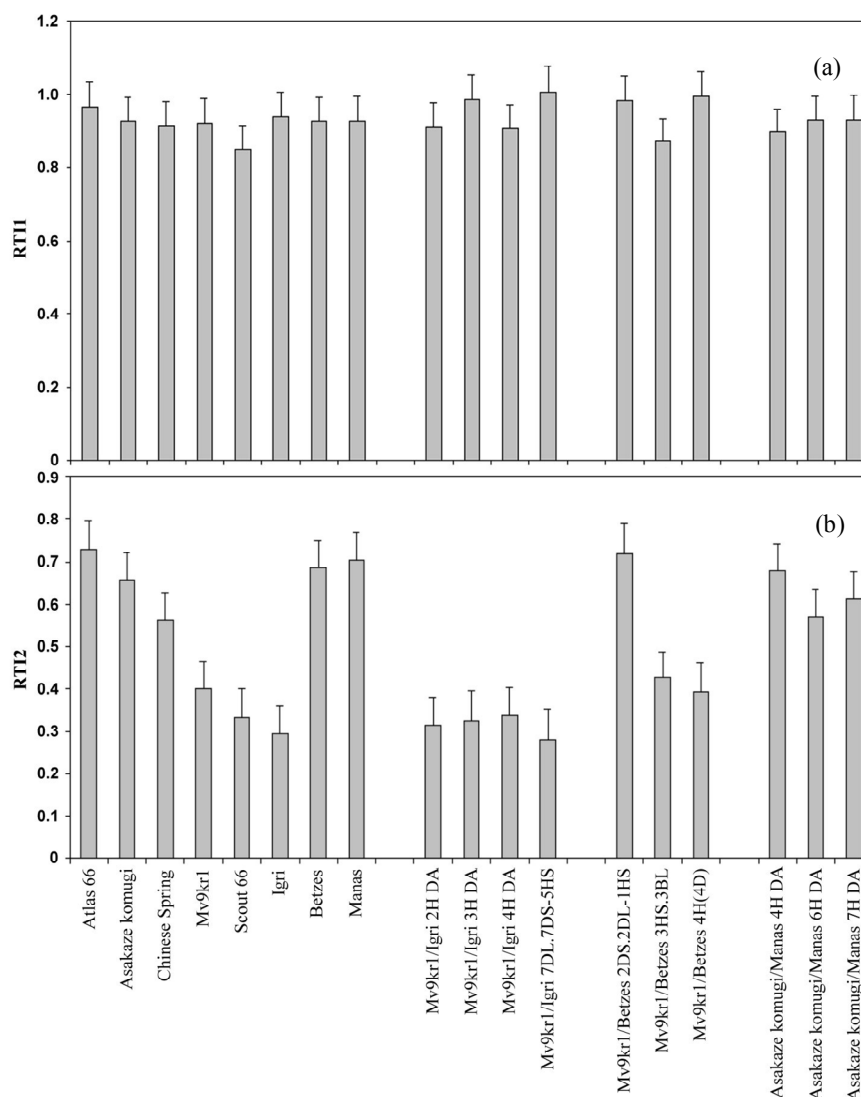


Figure 3. Root tolerance indexes of wheat and barley genotypes and wheat/barley introgression lines represents their tolerance to acid pH 4.0 (RTI1) (a) and to aluminium (RTI2) (b). RTI values are calculated as described in Materials and Methods. Data are means \pm SD of three independent experiments.

exhibited relatively low values in comparison with RTI1 and varied over a much wider range from 0.295 to 0.728 (**Figure 3(a)**), indicating considerable diversity in the Al tolerance of both the wheat and barley parental genotypes. The RTI2 values of the wheat/barley introgression lines were similar to those found for the wheat parents, with one exception: the translocation line 2DS.2DL-1HS had the highest Al tolerance of all the genotypes and cross derivatives (**Figure 3(b)**).

Morin fluorescence was used to detect the Al content of root apices in the 2DS.2DL-1HS translocation line and to compare it to with that of the wheat Mv9kr1 and barley Betzes parents. Without Al, the fluorescence intensity of morin was relatively low (**Figure 4(a)**), while the Al-morin complex induced intense fluorescence emission (**Figures 4(b)-(d)**). A lower fluorescence yield was de-

tected in the roots of the 2DS.2DL-1HS translocation line of Mv9kr1/Betzes than that observed for the wheat and barley parents.

4. Discussion

The first symptom of Al toxicity is the inhibition of root growth. Culture in nutrient solution and staining processes (especially with haematoxylin) are common screening methods for the evaluation of the Al tolerance of different genotypes [13].

Wheat and barley genotypes grown in hydroponic solution at pH 5.5 and pH 4.0 with and without Al demonstrated different root growth potential, relatively good tolerance of acidic soil, but they exhibited a wide range of Al toxicity responses. This confirms that Al toxicity is

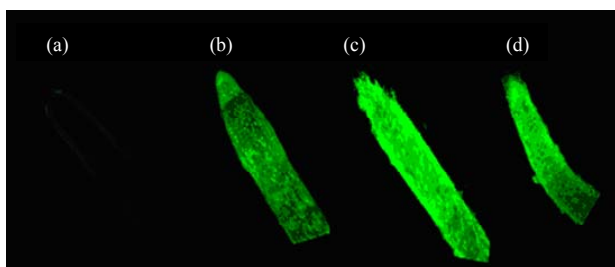


Figure 4. Al content of root apices of 2DS.2DL-1HS translocation line of Mv9kr1/Betzes (b) and of wheat Mv9kr1 (c) and barley Betzes (d) parents grown in nutrient solution containing 75 μM AlCl_3 at pH 4.0 determined by morin staining. Autofluorescence of morin in root apex (a) grown in nutrient solution at pH 4.0 without Al.

the main limiting factor for root the growth of these plants under acidic conditions. Although intensive root growth may be a useful agronomic character in relation to many abiotic stresses, such as drought or nutrient deficiency [27], it seems that a high growth rate, as found in the wheat Mv9kr1 and in its hybrid derivatives, did not affect the Al tolerance level. Nevertheless, the high root growth found in lines containing the 4H chromosome of barley, irrespective of the parents, indicated the presence of quantitative trait loci (QTL) for root elongation on chromosomes 4H. A similar association was found previously by Raman *et al.* [19], Jefferies *et al.* [28] and Ellis *et al.* [29] under different stress conditions.

When evaluating the Al tolerance level of these introgression lines and parental genotypes, similar results were found by monitoring root growth and by the haematoxylin test. These methods indicated wide genetic variation both in root growth and regrowth potential and in Al tolerance between the cultivars and introgression lines (**Figures 1-3**). The root tolerance indexes (RTI1 and RTI2) express differences between the genotypes in terms of tolerance to acid pH and to Al, independently of genetic variations in root growth [14,15]. The absolute values of RTI2 were strongly dependent on the experimental conditions (Al concentration, pH, composition of the nutrient solution), but it is generally accepted that higher values of RTI indicate greater Al tolerance. Maxin and Duta [11] established three Al tolerance groups: RTI2 above 0.7: high Al tolerance level, RTI2 0.4 - 0.7: medium tolerance and RTI2 below 0.4: low Al tolerance. In the present experiments, RTI2 was above 0.7 in the Al-tolerant wheat Atlas 66, 0.563 in the moderately Al-tolerant Chinese Spring and very low (0.333) in the Al-sensitive Scout 66, in agreement with previous results [30,31]. Comparing the results of root growth and the root regrowth test, it seems that the Al tolerance level of the six-rowed winter barley Manas is comparable to that of the winter wheat Atlas 66. Moreover, the Al tolerance of the facultative wheat Asakaze komugi and the two-rowed spring barley

Betzes was at least as high as that of the moderately Al-tolerant Chinese Spring (**Figure 3(b)**), making them suitable for cultivation in areas prone to soil acidity. The winter wheat Mv9kr1 and the two-rowed winter barley Igri proved to be the Al-sensitive genotypes.

Studies on the wheat/barley introgression lines showed that the parental genotypes of the introgression lines had similar Al tolerance. Lines originating from the cross between Mv9kr1 and Igri had Al tolerance as low as that of the parents. By contrast, lines originating from Asakaze komugi and Manas had high Al tolerance again similar to that of the parents. Due to the fact that there was no contrast in the Al tolerance of the parents of the wheat/barley addition lines the effect of the added barley chromosomes on Al tolerance was difficult to evaluate. In spite of the fact that the dominant genes (ALMT and MATE) responsible for Al tolerance (via the secretion of malic and citric acid) in wheat and barley are located on chromosomes 4D and 4H, respectively, the presence of 4H chromosomes did not result in elevated Al tolerance in any of the 4H disomic addition lines. This indicates that the barley genes were not manifested in a wheat background. This was confirmed by the results obtained for the 4H (4D) substitution line, where the Al resistance allele of barley Betzes was obscured in a wheat genetic background.

The Al tolerance level did not alter in the 3HS.3BL translocation line, containing a centric fusion of the wheat 3B and barley 3H chromosomes, as compared to wheat Mv9kr1, despite containing chromosome segments from the barley Betzes, which has a high level of Al tolerance. These results indicated that neither of these chromosome segments contains genes responsible for Al tolerance. In contrast, an elevated Al tolerance level was observed in the 2DS.2DL-1HS translocation line originating from wheat Mv9kr1 and barley Betzes, suggesting that either the lack of the distal part of the 2DL chromosome or the presence of the distal part of 1HS was able to improve Al tolerance. As indicated by morin staining, high Al tolerance was related to low Al content in the roots, showing that an exclusion mechanism operates in this line. The isolation and sequencing of chromosome 1H [32] may help to identify genes localized on the distal part of 1HS and to determine the role of 1HS in Al tolerance.

5. Conclusion

Our investigations demonstrated that the derivatives of the wheat Asakaze komugi and barley Manas hybrid have good tolerance to Al. In most cases, the alien chromosomes of the introgression lines tested were unaffected by the wheat background due to the fact that there was no contrast in the Al tolerance of the parents. However, the lack of the distal part of the 2DL chromosome of wheat or the presence of the distal part of 1HS from

barley improved the level of Al tolerance.

6. Acknowledgements

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