The influence of homeologous D(A) and D(B) substitutions on plant dry matter, nitrogen and phosphorus accumulation and utilization efficiency in hexaploid triticale young plants grown in hydroponics

Wpływ homeologicznych substytucji D(A) i D(B) w siewkach heksaploidalnego pszenżyta na gromadzenie suchej masy, akumulację oraz wykorzystanie azotu i fosforu w kulturze hydroponicznej

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The accumulation and utilization efficiency of nitrogen and phosphorus were studied using hydroponic cultures of seedlings of the ‘Presto’ and ‘Rhino’ D(A) and D(B) substitution lines of hexaploid triticale. The results were significantly affected by homoeology group of chromosomes participating in a substitution, the A or B genome origin of replaced chromosome and genetic background of triticale cultivar. The substitutions 4D(4B) and 5D(5B) resulted in an increase of plant dry matter in relation to the non-substituted cultivars. The significant increase of N accumulation was found in 3D(3A) and 4D(4B) of ‘Rhino’, and 5D(5B) and 4D(4B) of ‘Presto’. The improvement of N utilization efficiency was recorded for 2D(2A) and 1D(1B) of ‘Presto’, and 5D(5B), 7D(7A) and 5D(5A) of ‘Rhino’. The P accumulation was distinctly improved in ‘Presto’ 5D(5B) substitution. The P utilization efficiency was improved in all substitutions containing 4D or 6D as well as in ‘Presto’ 2D(2B) and 5D(5B).

Key words: breeding, D-genome, N accumulation, N utilization efficiency (NUE), P accumulation, P utilization efficiency (PUE), substitution lines, triticale

Introduction

Breeding of plants efficient in absorption and utilization of nutrients is an undervalued tool to improve efficiency of applied fertilizers, to reduce input costs and to prevent leaks of nutrients to the ecosystem. Large differences among species and cultivars in absorption, translocation and utilization of mineral nutrients are known (Baligar et al. 2001). Due to multigenic character of nutrient metabolism the control or manipulation of genes involved is difficult and results are unpredictable.

A simpler way to improve nutrient metabolism in alloploid cereals is manipulation on the chromosomal level using substitutions or translocations, which can result in efficiency of mineral fertilization (Górny 2000).

The hexaploid triticale (xTriticosecale Wittmack, genome formula AABBRR), due to presence of rye genome (R), is a species showing remarkable improvement in accumulation of nitrogen and phosphorus as compared to wheat (Triticum aestivum L. - AABBDD)
Material and Methods

The material consisted of two sets of disomic D(A) and D(B) substitution lines, derived from the hexaploid triticale cultivars 'Presto' (winter) and 'Rhino' (spring) (Łukaszewski, 1990). Both sets contained all possible homoeological substitutions of wheat D genome chromosomes for their A or B genome counterparts, except for the 7D (7B) in 'Rhino'. The non-substituted cultivars 'Presto' and 'Rhino' were used as controls.

The procedures of seed germination and planting, growth chamber parameters and the medium composition had been described in our earlier paper (Oracka and Łapiński, 2005). Seedlings were kept in 4°C for 30 days and then planted on plastic tanks containing 40 l of the modified Hoagland no. 2 solution (Hoagland, and Arnon, 1950) at 50% reduced level of mineral elements. The plants were grown in a growth chamber supplied with the fluorescent lamps (MASTER TLD 58W/865, PHILIPS), at irradiance of 350 mmol m⁻² s⁻¹ and 16h day length. The nutrient solution was aerated for 10 minutes every hour and renewed every 4 days. The temperatures were 18°C (day) and 13°C (night), air humidity was 70%.

The experiment was designed as four replicates of four plants for each line. The plants were harvested after 41 days of growth (shooting stage) and plant dry matter was determined (after drying overnight at 105°C). Standard procedures (Kjeldahl digestion, ammonium molybdate photometry) were used to determine N and P content in dry matter. The N and P accumulation was calculated from the dry matter and N, P concentrations, and the utilization efficiencies (NUE, PUE) were calculated as ratios of plant dry matter to total content of N or P in plants.

Analysis of variance (ANOVA) was performed to test the differences between objects for all parameters studied. Significant effects (P < 0.05) were explored using Tukey’s LSD procedure.

Results

The D(A) and D(B) chromosome substitutions caused a significant variability of the examined parameters, as shown in Table 1. The differences were dependent on cultivar, chromosome homoeology group and whether A or B genome chromosomes were substituted.

Plant dry matter of the 16 substitution lines was significantly lower than that of the cultivar standards, 9 lines showed a similar level and two ones exceeded significantly the original cultivar. The ‘Presto’ substitutions were generally more variable than those of ‘Rhino’. The lowest ‘Presto’ group results were recorded for the substitutions 4D(4A) and 1D(1A) and the highest ones for the substitutions 4D(4B) and 5D(5B). The 1D(1B), 2D(2A), 3D(3B), 4D(4A) and 6D(6B) substitutions negatively affected plant vigor, regardless the acceptor cultivar. The lacking 7D(7B) substitution would also be included to the abovementioned group, as the karyotype inviability on the ‘Rhino’ genetic background may be considered an extreme case of negative influence. Considering the relation of the substitution lines to their standard cultivars, the 2D(2B) and 5D(5A) on the ‘Presto’ background performed significantly better than in context of the ‘Rhino’ chromosomes. On the other hand, the 1D(1A), 3D(3A) and 7D(7A) performed better on the ‘Rhino’ genetic background. The 4D(4B), 5D(5B) and 6D(6A) did not cause significant decrease of plant dry matter in both cultivars.

Nitrogen accumulation: The amount of nitrogen absorbed was usually proportional to the plant dry matter content. The group of lines containing the 4D(4B) and 5D(5B) substitutions of ‘Presto’ and 3D(3A) 4D(4B) substitutions of ‘Rhino’ performed significantly better than standards. In relation to dry matter, the 7D(7A) ‘Presto’ substitution and ‘Rhino’ 3D(3B) showed increase of N accumulation.

Nitrogen utilization efficiency (NUE): The variability of NUE was much lower among the studied lines than in dry matter or N accumulation. However, significant superiority over the standard
The influence of homoeologous D(A) and D(B) substitutions on plant dry matter... cultivars was noted for the 1D(1B) and 2D(2A) of ‘Presto’ and 5D(5A), 5D(5B), 7D(7A) of ‘Rhino’. The lines 6D(6A) and 7D(7A) of both cultivars and 1D(1A) of ‘Rhino’ showed relatively high levels of nitrogen accumulation and efficiency of use, in spite of a negative correlation of these parameters in majority of the lines.

**Phosphorus accumulation** was highly variable, depending on cultivar and chromosome homoeology group. In majority of the ‘Presto’ substitution lines the P accumulation was distinctly lower than in the standard cultivar. The 5D(5B) line was the only positive exception, showing significant increase of P content, in relation to the standard cultivar. No significant decrease has been observed for the 4D(4B) ‘Presto’ substitution. In the ‘Rhino’ cultivar the negative effect of the substitutions on P accumulation was less pronounced and only half of the lines showed significant decrease in P accumulation, but no line was significantly better.

**Table 1**

**Effects of D-genome chromosomes’ substitutions in the ‘Presto’ and ‘Rhino’ triticale cultivars on plant dry matter and on uptake and utilization efficiency of nitrogen and phosphorus (NUE and PUE, respectively), LSD – lowest significant difference, (+) – significantly higher than control.**

| ‘Presto’ substitution | Dry matter [mg] | Nitrogen content [mg per plant] | NUE | Phosphorus content [mg per plant] | PUE |
|----------------------|----------------|-------------------------------|-----|----------------------------------|-----|
| non-substituted      | 748.1          | 24.94                         | 30.0| 7.03                             | 102.3 |
| 1D(1B)               | 544.6          | 17.83                         | 30.6(+)| 5.16                             | 101.8 |
| 2D(2A)               | 459.0          | 16.33                         | 31.2(+)| 4.53                             | 100.6 |
| 2D(2B)               | 802.5          | 25.65                         | 28.1| 5.72                             | 115.5(+)|
| 3D(3A)               | 502.0          | 16.61                         | 30.2| 4.70                             | 103.5 |
| 3D(3B)               | 525.0          | 17.84                         | 29.4| 5.16                             | 101.1 |
| 4D(4A)               | 351.5          | 11.65                         | 30.2| 3.26                             | 104.6(+)|
| 4D(4B)               | 831.8(+)| 28.14(+)| 29.6| 6.72                             | 115.8(+)|
| 5D(5A)               | 743.6          | 26.40                         | 28.2| 6.01                             | 107.3(+)|
| 5D(5B)               | 887.4(+)| 30.69(+)| 28.9| 8.03(+)| 103.3 |
| 6D(6A)               | 699.3          | 23.21                         | 30.1| 5.74                             | 109.3(+)|
| 6D(6B)               | 445.5          | 15.84                         | 28.1| 3.89                             | 109.5(+)|
| 7D(7A)               | 687.1          | 23.78                         | 28.9| 6.07                             | 101.8 |
| 7D(7B)               | 540.9          | 18.06                         | 29.9| 4.69                             | 101.1 |
| LSD                  | 55.2           | 1.90                          | 0.3 | 0.50                             | 1.3 |

| ‘Rhino’ substitution | Dry matter [mg] | Nitrogen content [mg per plant] | NUE | Phosphorus content [mg per plant] | PUE |
|----------------------|----------------|-------------------------------|-----|----------------------------------|-----|
| non-substituted      | 1391.6         | 47.61                         | 29.2| 14.86                            | 93.3 |
| 1D(1A)               | 1399.6         | 47.99                         | 29.2| 15.76                            | 81.0 |
| 1D(1B)               | 1181.7         | 40.41                         | 29.2| 13.81                            | 82.8 |
| 2D(2A)               | 934.5          | 33.03                         | 28.3| 9.66                             | 89.9 |
| 2D(2B)               | 1215.8         | 41.98                         | 29.0| 13.04                            | 91.7 |
| 3D(3A)               | 1475.0         | 52.94(+)| 27.9| 15.79                            | 88.9 |
| 3D(3B)               | 1253.4         | 43.41                         | 28.9| 13.71                            | 84.2 |
| 4D(4A)               | 958.4          | 33.37                         | 28.7| 8.38                             | 103.7(+)|
| 4D(4B)               | 1488.4         | 52.15(+)| 28.5| 13.35                            | 103.8(+)|
| 5D(5A)               | 1070.8         | 36.42                         | 29.4(+)| 11.12                            | 93.5 |
| 5D(5B)               | 1387.3         | 44.23                         | 31.4| 15.04                            | 91.9 |
| 6D(6A)               | 1457.9         | 50.29                         | 29.0| 14.51                            | 94.9(+)|
| 6D(6B)               | 807.5          | 29.96                         | 27.0| 8.20                             | 96.4(+)|
| 7D(7A)               | 1396.2         | 46.12                         | 30.3(+)| 14.83                            | 90.1 |
| LSD                  | 126.2          | 4.30                          | 0.1 | 1.20                             | 0.8 |
Phosphorus utilization efficiency (PUE): Variability of the parameter was noticeably higher than for NUE and number of lines with positive reaction was higher than for the other analyzed indices. Only two lines of ‘Presto’, namely 1D(1A) and 2D(2A), showed significantly lower PUE than the cultivar standard, while six ones: 2D(2B), 4D(4A), 4D(4B), 5D(5A), 6D(6A) and 6D(6B) were significantly more effective. The majority of substitutions in ‘Rhino’ showed a significant negative effect, while 4D(4A), 4D(4B), 6D(6A) and 6D(6B) substitutions caused a significant PUE increase. The PUE for 5D(5A) line was on the level of the ‘Rhino’ standard.

Discussion

The most interesting substitutions which result in improvement of N and P management in the triticale seedlings proved to be 5D(5B) and 4D(4B). The plant vigor was not reduced, in contrast to majority of other chromosome replacements. In ‘Presto’ the increases of dry matter, in relation to that of standard cultivar, were significant (by ca. 19% and 11%, respectively). Accumulation of nitrogen and phosphorus was mainly a function of plant dry matter (the correlation coefficients $r = 0.996$ and $r = 0.977$, respectively). The NUE and PUE parameters were correlated negatively with the dry matter ($r = -0.183$ and $r = -0.634$, respectively), however more variation was left for the more specific effects. The highest increase of NUE has been noted in the ‘Rhino’ 5D(5B) – by about 7%. The comparison of performance of 5D(5B) and 5D(5A) lines suggests importance of the 5D per se in ‘Rhino’ cultivar, while in ‘Presto’ cv. significance of replaced A or B chromosome can be seen. The PUE values for 4D(4B) in both ‘Presto’ and ‘Rhino’ substitutions were significantly higher than in the standards (by ca. 13% and 11%, respectively). The lowest level of plant vigor and N and P accumulation in the 4D(4A) substitutions of both sets can be attributed to large structural differences between the 4D substituting and the 4A substituted chromosomes due to the known 4AL-5AL and 4A-7B heterologous translocations (Naranjo et al., 1987, Cao et al., 1989, Nelson et al., 1995). Thus, the high usefulness of 4D could be expressed only in the 4D(4B) substitutions. Probably the same cause makes production of the ‘Rhino’ 7D(7B) line difficult. The highest PUE seems to be correlated with presence of the 4D rather than with absence of 4A or 4B. Our previous investigation on the D(R) substitutions (Oracka, Łapiński, 2006) indicated extremely positive effects of the 4D”(4R”) incomplete substitution (with one rye chromosome maintained) in ‘Rhino’ on the parameters studied, which confirms high value of the 4D chromosome in engineering of environment friendly triticale. In the research of Budzianowski and Woś (2004) the chromosomes 4D, in the same 4D(4A) and 4D(4B) substitution lines, were among the most efficient in rising tolerance to high concentration of aluminium ions.

The highest accumulation of the nutrients has been found in the 3D(3A) line of ‘Rhino’, together with high scores for plant dry matter. It is inconsistent with the results in our paper mentioned above, which shows that the ‘Presto’, not the ‘Rhino’ gene background contributes to the 3D chromosome superiority in the N and P accumulation when substituted for the 3R. The difference cannot be attributed to different allelic composition of the 3D chromosome, which was introduced from the wheat cultivar ‘Grana’ (Lukaszewski 1990, Budak et al. 2004). The 6D(6A) substitution of ‘Rhino’ did not show as significant and complex effect on nutrients management as the 5D(5B) and 4D(4B), however the PUE level was slightly increased without negative side effects on plant dry matter and other parameters studied. The PUE parameter also increased in 6D(6B), but the plant vigor was much lower. The results for both 6D(6A) and 6D(6B) substitutions indicate that presence of the 6D chromosome rather than absence of the 6A or 6B is responsible for PUE increase. The 6D(6R) substitution of rye chromosome (from our earlier studies) also exerted positive effect on the PUE and NUE parameters (Oracka, Łapiński, 2006). The PUE and plant dry matter were remarkably high also in the 2D(2B) substitution of ‘Presto’. The low values in ‘Rhino’ 2D(2B) show importance of differences in varietal background. The possible influence of different 2D allelic composition has to be excluded here, because in production of the ‘Presto’ set the substitutions of ‘Rhino’ served as donors and the same variants were used as donors, which shows that the ‘Presto’, not the ‘Rhino’ gene background contributes to the 3D chromosome superiority in the N and P accumulation when substituted for the 3R. The difference cannot be attributed to different allelic composition of the 3D chromosome, which was introduced from the wheat cultivar ‘Grana’ (Lukaszewski 1990, Budak et al. 2004). The 6D(6A) substitution of ‘Rhino’ did not show as significant and complex effect on nutrients management as the 5D(5B) and 4D(4B), however the PUE level was slightly increased without negative side effects on plant dry matter and other parameters studied. The PUE parameter also increased in 6D(6B), but the plant vigor was much lower. The results for both 6D(6A) and 6D(6B) substitutions indicate that presence of the 6D chromosome rather than absence of the 6A or 6B is responsible for PUE increase. The 6D(6R) substitution of rye chromosome (from our earlier studies) also exerted positive effect on the PUE and NUE parameters (Oracka, Łapiński, 2006). The PUE and plant dry matter were remarkably high also in the 2D(2B) substitution of ‘Presto’. The low values in ‘Rhino’ 2D(2B) show importance of differences in varietal background. The possible influence of different 2D allelic composition has to be excluded here, because in production of the ‘Presto’ set the substitutions of ‘Rhino’ served as donors and the same variants of D-chromosomes are present in the respective lines of both sets (Lukaszewski, 1990). High importance of the 2D chromosome in determination of all parameters studied is confirmed by our earlier results on the D(R) substitutions in triticale, where the NUE and PUE reach the highest levels in the 2D(2R) line (Oracka, Łapiński 2006). The highest 2D(2A) value for NUE in ‘Presto’ in the present paper is in agreement with those results.

The 1D(1A) and 7D(7A) ‘Rhino’ substitutions are also worth of mention as representing relatively high levels of all studied parameters, except for PUE. The respective ‘Presto’ substitutions performed much worse, suggesting that some
reserves may exist in recombination between the studied cultivars, even without the D-genome manipulation.

The results presented above show the importance of cultivar genetic background for effective substitution. Comparison of respective ‘Presto’ and ‘Rhino’ substitution lines suggests that much more variation is to be discovered in triticale after creation of substitution lines from different sources of A, B and D genomes. A reduction of viability is a rule in substitutions, even on the 6x ploidy level, well buffered by genetic redundancy. The increases of dry matter in ‘Presto’ 5D(5B) and 4D(4B) seem to contradict this tendency.

However, the field trials performed by Budak et al. (2004) with the same ‘Presto’ substitution set did not show yield increase in any lines, including those deduced as promising from the presented lab experiment. The discrepancy may be attributed to the environmental circumstances, much different in field than in phytotron hydroponic culture. Another source of differences may be the growth phase difference, the N and P metabolism in young plants studied here is not necessarily the same as in the more advanced growth stages. Nevertheless, the results confirm the main idea on the potential for improvement of nutrient management in the D(A) and D(B) substitution lines of hexaploid triticale. The identification of most promising substitutions seems also worth of attention, because in our earlier studies the interspecific differences between wheat and triticale, concerning the same parameters studied in the same way on seedlings in phytotron hydroponic, were in agreement with those observed in field practice.

We do not agree with the final suggestion of Budak et al. (2004), that no major benefit can be expected from the D-genome substitutions. Such conclusion cannot be valid as based on the unbalanced cytogenetic stocks where no effort has been put in breeding adjustment of a proper gene background to a particular substitution. Thus, only the bred forms of substituted 2D(2R) spring triticales of Mexican origin show high yield potential, particularly on alkaline soils, where they outyield the non-substituted forms with complete rye genome (Royo et al. 1993). Considering that evolutionary distance between the D genome and its A or B relatives is lower than between the D and R genomes, one could expect less difficulties in breeding adjustment and development of a D(A) or D(B) whole chromosome substitution cultivar.

As known from wheat breeding, chromosome arms have more chances for successful assimila-

tion into breeding populations than whole alien chromosomes. The 1RS.BL and 1RS.AL translo-
cations in bread wheat are the examples of such chromosome arm replacement resulting from the frequently made triticale-wheat crosses. The 1RS arm was spontaneously and widely included into the breeding populations because of its effect on adaptation to adverse environmental conditions (Lukaszewski 1990b, Hoffmann 2008). Crosses between whole chromosome substituted and non-substituted forms produce relatively frequent centromeric translocations (Friebe et al. 2005). The same could be expected for the chromosome arms of D-genome positively affecting management of mineral nutrients.

Introduction of the D-genome chromosomes into hexaploid triticale is worth of effort considering other possible advantages detected by other authors. The 5D chromosome in triticale controls better frost tolerance (Sutka and Snape, 1989) and increases grain hardness (Campbell et al. 1999). In the research of Budzianowski and Woś (2004) the chromosomes 4D and 6D, from the same sets of substitution lines, were among the most efficient in rising tolerance to high concentration of aluminum ions. Another advantage of the 6D incorporation into triticale may be improvement of baking quality (Payne, 1987). Additionally, the presence of D-genome chromosomes in triticale could enable access, via the Triticum aestivum bridge, to wide variation of the wild D-genome progenitor – Triticum tauschii, which is being recently extensively explored as a source of biotic and abiotic stress resistance for bread wheat (Mujeb-Kazi 2008). The T. tauschii is mentioned also by Huang et al. (2007) as a species containing genes responsible for high nutrient utilization efficiency.

Conclusions

The results confirm usefulness of homeologous substitution of whole chromosomes as a tool for improvement of multigenic traits in allopolyploid species.

The 4D(4B) substitution proved to be most effective in improvement of nitrogen and phosphorus management in Rhino (winter) cultivars of hexaploid triticale, whereas 3D(3A) and 5D(5B) positive effects were observed in Rhino and Presto, respectively.

The effects of particular chromosome substitutions were frequently specific to the cultivar sets in which they were present.
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