

BRIEF COMMUNICATION

Response of barley lines with structural rearrangements in chromosomes 5, 6 and 7 to limited nitrogen nutrition

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The responses of barley (*Hordeum vulgare* L.) lines with rebuilt chromosomes 5, 6 and 7 to reduced nitrogen nutrition were evaluated in juvenile growth stages. The material included two series of duplications (D) produced in the short arm of chromosome 6 and of chromosome 7, and in the long arm of chromosome 5 and of chromosome 6; their parental translocation lines (T) – from which analyzed duplications were derived and a standard karyotype cv. Bonus as a control. The translocation lines have break points located in 6_S and 7_S, or 5_L and 6_L. Only the lines with duplicated segments of the short arms of satellited (6 and 7) chromosomes exhibited an improved tolerance to reduced nitrogen supply. No changes relative to cv. Bonus were observed in the T-lines. More tolerant D-lines showed lower stimulation of the root development. Obtained results suggests that the adaptability factors for the low N tolerance at the vegetative growth stage of barley are located in the short arms of 6 and 7 chromosomes.

Additional key words: duplications, *Hordeum vulgare*, stress tolerance.

Translocation-derived duplications with varying lengths of the duplicated segments of chromosome arms may be a suitable material to study the dose effects of certain chromosome segments on the physiological functions of a plant (Hagberg and Hagberg 1986). Between others, they may also permit the allocation to chromosome arms of the genetic factors responsible for barley tolerance to water and mineral imbalances. The large collection of translocation lines described in a series of papers as Hagberg *et al.* (1978) was the basic material for direct production of duplications

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of chromosome segments. The first set of such duplication lines of barley was developed from translocations involving the cytological markers (chromosomes carrying the satellites) (Hagberg *et al.* 1978, Hagberg *et al.* 1983, Hagberg and Hagberg, 1978, 1991). Later on another set of duplication lines with duplicated segment of chromosomes 5 and 6 in barley was selected (Sodkiewicz *et al.* 1996, 1998).

Plant response to insufficient mineral nutrition appears to be complex and consists of various morpho-physiological mechanisms (Sarić 1983, El Bassam *et al.* 1990, Górny 1996). Different patterns of such responses suggest a high complexity of their genetic background. At present, little is known on chromosomal location of the genes involved in such phenomena. As described previously (Sodkiewicz 1993), duplicated barley lines exhibited a lower vigour and reduced yield potential under field conditions. However, in some cases double doses of certain chromosome segments were associated with higher plant biomass and grain yield relative to the normal karyotype (Hagberg and Hagberg 1986). According to Hagberg and Hagberg (1991), such a basic material could be a challenge between cytological and genetic maps of diploid barley chromosomes.

The purpose of the study was to examine the effects of different duplications and translocations involving chromosomes 5, 6 and 7 on the shoot and root variation under varied nitrogen nutrition and to evaluate the effects of the chromosomal changes on barley tolerance to nitrogen limitations at the early growth stages.

Eight duplicated (D) lines, eight translocated (T) lines involving the break points in chromosomes 5 and 6 or 6 and 7 (parental forms for D-lines), and their parental barley (*Hordeum vulgare* L.) cultivar Bonus (as initial, unchanged karyotype) were included in the study. The lines carrying duplications consisted of two types: lines 6D-9D differ in the number and lengths of duplicated segments on the short arms of chromosomes 6 and 7, and lines D205/2-D207/2 differ respectively in duplicated segments of long arms of 5 and 6 barley chromosomes. Details on the location and the size of duplicated segments as well as idiograms of the duplications were discussed previously (Sodkiewicz 1993, Sodkiewicz and Sodkiewicz 1994, Sodkiewicz *et al.* 1996, 1998). The parental reciprocally translocated T6-7 lines used for direct producing duplications (according to the method suggested by Burnham and Hagberg in 1956), have interchanged chromosomes 6_S and 7_S which are distinguishable from each other; the breakpoints positions in these chromosomes were located based on cytogenetic observations (Hagberg *et al.* 1978). The parental translocations T5-6 has interchanges involving chromosome 5_L and 6_L which are distinguishable from one another; the breakpoint positions were located based on observations of Hagberg *et al.* (1975).

Two factorial completely randomized experiments with nine genotypes, at two N nutrition levels and three replications were two times performed. Plants were grown in 30 cm long filter paper rollers (Górny 1992) in a climatic chamber under 16-h photoperiod, irradiance of 200 - 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperatures 20 - 22/14 - 16 °C and relative humidity 60 %. Ten uniform and pregerminated seeds were sown in each of the roller-replication. Packets were vertically placed in a shallow bath with nutrient solutions changed three times per week. To overcome the

possible effects of light and temperature gradients, the roller packets were frequently displaced around the experimental area. In two repetitions of the experiment, the modified Knop's and half-strength Hoagland's solutions (pH 6.2) were used with high N (control) and low N concentration. The Knop's solutions contained 8.4 mM N (control) and 0.4 mM N (low N). The Hoagland's solutions contained 5.0 mM N (control) and 0.5 mM N (low N). Plants were harvested at the 2-leaf stage. Shoot dry matter (d.m.), total root length, rooting depth and the number of main root axes were measured. Index of the low N tolerance (T_s) was estimated for the shoot dry matter using the following equation: $T_s = [L/H]/D$, where L is the shoot d.m. at the low nutrition, H is the shoot d.m. at the high nutrition, and the D value is a measure of the mineral stress (equals to the mean L-value averaged over all genotypes divided by the mean H value averaged over all genotypes). *MSTAT-C* package was used for the statistic data computations.

All duplicated D(6-7) lines accumulated more dry matter in shoots than the cv. Bonus (Table 1). Vigorous shoot growth of the duplications in short arms of 6 and 7 chromosomes was accompanied by poorer rooting. Relative to the cv. Bonus, no such alterations in the shoot and root growth were apparent in most of the lines with translocations of 6 and 7 chromosomes (T6-7). The T-lines had a higher number of the main root axes (only the 1T-line formed a considerably less vigorous root system). The genotypes with rearrangements in short arms of 6 and 7 chromosomes differed in the tolerance to reduced nitrogen. In relation to the unchanged Bonus genotype no changes in the low N tolerance were observed in the T-lines; but lines carrying duplications of segments of 6S and 7S chromosome arms showed enhanced

Table 1. Shoot and root characteristics and the index of tolerance to reduced nitrogen (T_s) of spring barley (*Hordeum vulgare*) genotypes with rebuilt karyotype in 6 and 7 chromosomes at the seedling stage.

	T_s	Shoot d.m. [mg plant ⁻¹]		Total root length [cm plant ⁻¹]		Rooting depth [cm]		Number of main root axes [plant ⁻¹]	
		control	low N	control	low N	control	low N	control	low N
cv. Bonus	0.91	22.9	17.0	108.9	141.2	21.5	27.3	6.85	6.38
Line 1T	0.93	23.0	16.6	94.1	118.0	18.6	22.1	6.60	7.00
Line 2T	0.91	21.9	17.0	109.7	141.0	22.2	26.4	6.52	6.93
Line 3T	0.94	24.6	16.8	105.9	139.2	21.6	26.6	6.53	6.95
Line 4T	0.93	24.6	18.6	117.7	149.5	23.3	27.6	6.66	6.93
Line 6D	1.10	23.1	20.6	77.8	92.5	16.7	18.1	6.00	6.00
Line 7D	1.04	20.3	21.7	112.9	140.5	23.9	27.7	6.33	6.32
Line 8D	1.09	19.9	18.0	101.6	129.2	18.7	23.1	6.93	6.88
Line 9D	1.17	22.1	19.0	102.6	125.1	20.2	24.0	6.54	6.58
LSD _{0.05}		2.2		8.3		1.3		0.33	

tolerance for the limited nitrogen nutrition relative to the translocated lines and to the original cv. Bonus (Table 1). The highest tolerance to low N was observed in line 9D which has the highest per cent of duplicated chromosome sectors.

Relative to the high N, cv. Bonus accumulated about 26 % less shoot d.m. under low N nutrition, while the shoot d.m. of the lines with duplications of 6S and 7S chromosome arms was reduced only by 5 - 15 %. The reduction of the shoot d.m. of the T6-7 lines under low N was similar or somewhat stronger than that in cv. Bonus itself. Simultaneously, all these genotypes under low N formed more vigorous root systems than under the control nutrition. The stimulative effects of the low N supply on the root length were lower in the lines carrying duplications of 6S and 7S arms than in the initial cv. Bonus. The poorer was the root system, the higher was the low N tolerance ($r = -0.515$). The genotypes which accumulated more dry matter in shoots tended to be less susceptible to limited N nutrition.

Table 2. Shoot and root characteristics and the index of tolerance to reduced nitrogen (T_s) of spring barley (*Hordeum vulgare*) genotypes with rebuilt karyotype in 5 and 6 chromosomes at the seedling stage.

	T_s	Shoot d.m. [mg plant ⁻¹]		Total root length [cm plant ⁻¹]		Rooting depth [cm]		Number of main root axes [plant ⁻¹]	
		control	low N	control	low N	control	low N	control	low N
cv. Bonus	0.99	27.0	21.2	129.7	137.0	24.6	26.0	6.6	6.50
Line T(19)	0.94	24.4	18.4	115.7	116.8	24.1	23.3	6.5	6.40
Line T(15)	1.02	24.0	19.5	127.5	130.6	24.4	25.7	6.5	6.30
Line T(13)	1.11	24.2	21.2	121.4	115.8	24.7	23.3	6.4	6.33
Line T(10)	0.98	25.9	20.1	119.2	134.4	24.9	25.9	6.3	6.33
Line D205/2	0.98	24.8	19.4	117.2	118.8	24.7	24.5	6.0	6.17
Line D207/1	0.99	23.2	18.3	111.7	106.8	24.2	22.6	6.3	6.00
Line D207/2	0.92	23.4	17.2	117.3	123.0	24.6	25.3	6.3	6.27
Line D 206	0.95	25.4	19.3	119.8	116.9	24.7	23.7	6.2	6.13
LSD _{0.05}		2.9		12.4		2.0		0.49	

In the contrast, no variation in shoot and root indices among barley karyotype with rebuilt long arms of 5 and 6 chromosomes were observed (Table 2). At low N nutrition the shoot dry matter of lines with duplications of 5L and 6L arms tended to be smaller than in respectively translocation (T5-6) lines and cv. Bonus but stress/control ratios are very similar in all these genotypes. The same could be seen in data concerning total root length and number of main root axes (Table 2).

Average rooting depth was similar in cv. Bonus and in translocated (T5-6) as well as duplicated (D5-6) lines. The reactions to stress assessed were also consistent.

Strong differences observed in reactions to low N supply between group of genotypes with translocations and duplications of 6S and 7S chromosome arms (showing considerable variation of response) and group of genotypes with rearrangements of 5L and 6L chromosome arms (showing consistent reactions with cv. Bonus) give evidence that in duplicated segments of 6 and 7 chromosome there is a gene/or genes affecting barley response to nitrogen limitations. Duplication of this locus (loci) resulted in higher tolerance.

The characteristic response to low N nutrition is enhanced rooting. However, the genotype ability to develop poorer root system and accumulate more dry matter in shoots positively influenced low N tolerance.

The above mentioned results showed that comparative analysis of barley plants with translocation and duplication rearrangements in chromosomes could be the suitable method for location of genes influencing plant response to physiological stress.

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