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Single nucleotide polymorphism markers linked to root elongation rate in sugar beet

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Abstract

The aim of this study was to identify single nucleotide polymorphism (SNP) markers genetically linked to root elongation rate (RER) in sugar beet (*Beta vulgaris* L.). A population of 244 F_3 individuals, obtained from the cross between lines L01 (a low RER) and L18 (a high RER), was phenotyped by measuring RER of 11-d-old seedlings grown in a hydroponic culture. Two DNA bulks of 50 F_3 individuals with extreme phenotypes were used for bulk segregant analysis by restriction-associated DNA sequencing. A total of 20 376 SNPs were identified. Single nucleotide polymorphisms were filtered to reduce the number of the false positive and mapped on candidate chromosomal regions of the *B. vulgaris* reference genome. One of the total of SNPs selected, SNP10139, was strongly linked to RER ($P < 0.01$). The pattern of association between the SNP10139 genotype and RER was also evaluated on a breeding line panel comprising 40 low and 40 high RER individuals with different allele frequencies between groups ($P < 0.01$). The SNP10139 sequence was mapped on the *B. vulgaris* peptide transporter (*PTR*) gene, a carrier that influences root elongation in *Arabidopsis thaliana*. Our results suggest that SNP10139 influence RER in sugar beet, and sequence information can be used in marker-assisted selection programs.

Additional key words: abiotic stresses, *Beta vulgaris*, bulk segregant analysis, restriction-associated DNA sequencing.

Introduction

Roots play a central role in water and nutrient acquisition, and root characteristics involved in these functions are closely associated with crop productivity (Lynch *et al*. 1995). The ability of a plant to absorb nutrients distributed in the soil is given by the morphology of its root system. The improvement of root characteristics is essential to increase crop yield especially in environments subjected to recurrent water and nutritional stresses (De Dorlodot *et al*. 2007).

Lynch (2013) proposed a maize ideotype, termed "Steep, Cheap, and Deep", for superior nutrient and water acquisition. Root traits influencing rapid soil exploitation, such as root elongation rate (RER), could be used to develop crops with a greater water and nutrient acquisition (Lynch *et al.* 2014, Saengwilai *et al.* 2014). A recent study of sugar beet highlighted that key root traits, as RER, total length, surface area, and number of tips, are strictly related to sulfate acquisition and sugar beet yield (Stevanato *et al*. 2010). A significant and positive correlation was demonstrated between yield and nitrogen uptake rate in sorghum, or sulfate uptake rate in maize and sugar beet (Cacco *et al*. 1980, Saccomani *et al*. 1981, Nakamura *et al*. 2002, Stevanato *et al*. 2014).

The improvement of root traits through conventional breeding methods is slow because they are controlled by multiple gene loci (De Dorlodot *et al*. 2007), but with the assistance of molecular markers, a faster genetic control and improvement of a plant root apparatus can be achieved. Marker-assisted selection allows to determine major loci controlling root traits in rice (Courtois *et al*. 2003) and soybean (Liang *et al*. 2010). Root morphology is controlled by many genes, which interact with the environment, and were found to explain up to 30 % of phenotypic variation (Price *et al*. 2002, Giuliani *et al*. 2005). Similarly, Tuberosa *et al*. (2002) identified quantitative trait loci influencing root architecture and yield in maize. Single nucleotide polymorphism (SNP) markers have gained popularity in crop breeding programs by increasing the efficiency and accuracy of selection procedures (Ganal *et al*. 2009). Single nucleotide polymorphisms are ideal markers for identifying genes associated with useful traits in crops

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Abbreviations: BSA - bulk segregant analysis; RAD - restriction-associated DNA sequencing; *PTR* - peptide transporter gene; RER - root elongation rate; SNP - single nucleotide polymorphism.
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because they are abundant and densely located in plant genomes. The application of next-generation sequencing technology greatly facilitates a high throughput SNP discovery, and a large number of commercial platforms are available for automated SNP genotyping (Gupta *et al*. 2008).

Bulk segregant analysis (BSA) is method for identifying DNA markers linked to genes or genomic regions of interest (Michelmore *et al*. 1991). Samples of DNA from individuals showing a contrasting phenotype are compared with a large set of molecular markers to identify linkage to the trait of interest. This procedure has been successful in detection of major genes implicated in lateral root growth in rice (Wang *et al*. 2006) or root development in response to aluminum stress in barley (Raman *et al*. 2002) and wheat (Cai *et al*. 2008).

The objective of this study was to identify SNP markers linked to RER in sugar beet by means of BSA and to map SNP sequences to the reference *B. vulgaris* genome to identify candidate genes influencing root elongation.

Materials and methods

Plants: A population of 244 F_3 individuals, obtained from the cross between lines L01 (a low RER; 1.7 mm d^{-1}) and L18 (a high RER; 20.5 mm d^{-1}), was phenotyped by measuring RER of 11-d-old seedlings grown in a hydroponic culture. The F_3 plants were derived from a single F_1 individual and by a single-seed descent of 244 F_2 plants grown at the University of Padova (Italy). The pattern of association between the genotypes and RER was also evaluated on 80 individuals of an F_2 population, named F290, showing a wide variation of RER and provided by *Lion Seeds* (Maldon, UK).

Root elongation rate analysis: Seeds were surface-sterilized by immersion in 1 % (m/v) sodium hypochlorite, rinsed several times with distilled water, and then imbibed in aerated, deionized water at 22 °C for 12 h. The seeds germinated between two layers of filter paper moistened with distilled water in Petri dishes at 25 °C in the dark for 48 h. Only 3-d-old seedlings with 10 ± 2 mm long seminal roots were transferred into hydroponic plastic tanks with an aerated nutrient solution containing 200 μM $Ca(NO_3)_2$, 200 μM KNO_3, 200 μM $MgSO_4$, 40 μM KH_2PO_4, and microelements (Arnon and Hoagland 1940). The nutrient solution was replaced daily. The tanks were placed in a growth chamber set at day/night temperatures of 25/18 °C, a relative humidity of 70/90 %, a 14-h photoperiod, and an irradiance of 60 W m^{-2}. Primary root length of individual seedlings was manually measured each day until the seedlings were 11-d-old. Root elongation rate was calculated as the difference in root length between two measurements with the *WINRHIZO Pro* software (*Regent Instruments*, Quebeck, Canada). Trait distribution was tested for normality with Shapiro-Wilk test (Conover 1980).

Single nucleotide polymorphism discovery by restriction-associated DNA sequencing and bulk segregant analysis: DNA was isolated from 20 mg of leaf tissue with a *BioSprint 96* DNA plant kit in a *BioSprint 96* workstation (*Qiagen*, Hilden, Germany) following the manufacturer's instructions. It was assayed for concentration and purity by microfluidic gel electrophoresis with an *Agilent 2200 TapeStation* system (*Agilent Technologies*, Palo Alto, CA, USA). Based on F_3 sample phenotyping analysis, DNA of 50 individuals with extremely low and high RERs were selected for BSA restriction-associated DNA (RAD) analysis (*Floragenex*, Portland, Oregon, USA) following the methods outlined by Pegadaraju *et al*. 2013. Briefly, 100 bp paired-end *Illumina* sequences were obtained from the bulks. Restriction enzyme-derived reads were first trimmed to remove low quality sequences with an average phred-scaled quality score below 25 and then collapsed into RAD clusters sharing a complete sequence identity across the sequence flanking the restriction site. Only sequences with coverage between 20× and 1000× were considered in the analysis. The paired-end sequences were extracted for each RAD cluster passed to the *Velvet* sequence assembler for contig assembly and then aligned using *Bowtie* allowing up to three base pair mismatches between the paired-end read and the reference. Sequence variants were then followed using *SAM* tools. To provide a genomic anchor and location for the newly discovered SNPs, the RAD cluster sequences were aligned and mapped on the sugar beet reference genome (*v. RefBeet-1.1*; http://bvseq.molgen. mpg.de) using *BLASTN* (*v.2.2.27*) and allowing for a maximum of a single mismatch. For putative gene identification, some selected sequences were analyzed against the *Arabidopsis* genome database (*TAIR v. 10*) *TBLASTX* using a maximum threshold E-value of 10^{-10} (Altschul *et al*. 2010).

Linked-SNP validation by genotyping: From the SNP discovery analysis, all candidate SNPs were selected for validation on the 100 F_3 samples with extreme phenotypes used for BSA and on 80 F_2 samples with different RERs from a breeding population. Single nucleotide polymorphism genotyping was performed using the *QuantStudio 12K Flex* real-time PCR system and *OpenArray* technology (*Life Technologies*, San Diego, CA, USA) following the manufacturer's instructions. Briefly, 10 ng of DNA was mixed with 2.5 mm^3 of *TaqMan OpenArray Genotyping Master* and amplified. The results were analyzed using the *Taqman Genotyper* software (*v. 1.0.1*) and χ^2-test was adopted for association analysis between phenotypic and genotypic data.

Results

Root elongation rate evaluated on 244 individuals of the F_3 progeny showed a normal distribution (Wilk-Shapiro test for normality = 0.979; $P < 0.01$) with a certain degree of transgressive segregation for high values of RER (Fig. 1). Bulks were obtained from 50 F_3 individuals with the most extreme phenotypes; low and high RER bulks were characterized by average RERs of 6.3 ± 1.3 and 40.0 ± 8.9 mm d^{-1}, respectively (Fig. 1).

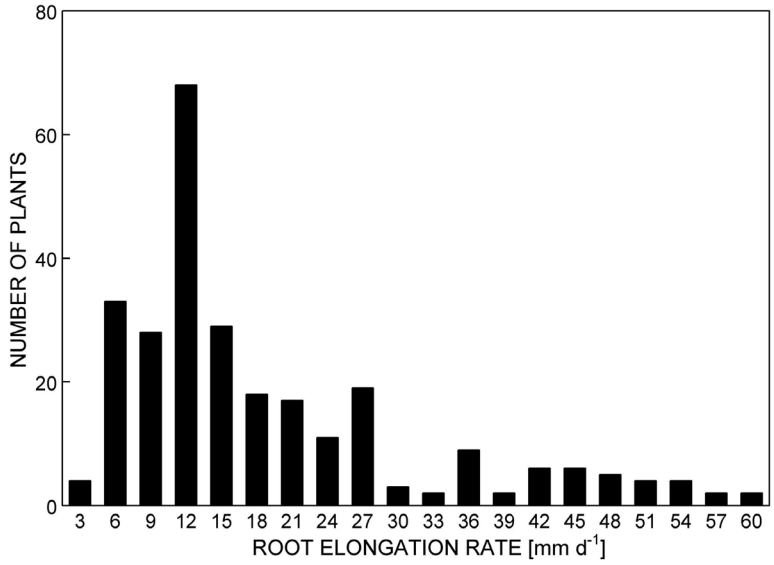

Fig. 1. Frequency distribution of root elongation rate (RER) in 244 F_3 individuals from the cross between a low RER L01 and a high RER L18 sugar beet lines.

Table 1. Frequency of the number of single nucleotide polymorphisms (SNPs) identified per sequence. The total number of sequences is 14 459.

Number of SNPs per sequence	Number of sequences	Percentage
1	9872	68.28
2	3403	23.53
3	1056	7.30
4	111	0.77
5	16	0.11
6	1	0.01

Illumina RAD sequencing yielded 68 360 881 raw reads of high quality with an average length of 102.4 nt. Overall, a total of 20 376 SNPs were identified on 14 459 alignments (Table 1). Most of the sequences presented a single SNP, whereas the remaining showed two or more SNPs in the same sequence. The majority of SNPs were diallelic (98.8 %) and more transitions (12 378) than transversions (7 746) were observed (a 1.6 ratio).

In order to reduce a false positive SNP association, appropriate quality filters were adopted; only sequences harboring single and diallelic SNPs were selected and then aligned to the sugar beet reference genome. For association between allele frequencies and RER phenotypes, only SNPs with a similar sequence coverage between bulks were selected (< 20 % coverage difference). Allele frequency ratios between bulks were estimated and only ratios higher than 2 (or lower than 0.5) were considered as candidate SNPs linked to the RER trait. A total of 234 SNPs passed the quality and association criteria and were selected as candidate markers associated to the RER trait.

The selected 234 SNPs were distributed across all 9 sugar beet chromosomes and located on 78 scaffolds (Table 2). Sequences of SNPs and their corresponding mapping coordinates are reported in Table 1 Suppl. The highest number of SNPs was observed in chromosome 8 (56) and the lowest in chromosome 7 (4). Among scaffolds with multiple mapped SNPs, the Bvchr8.sca002

Table 2. Distribution of 234 selected single nucleotide polymorphisms (SNPs) putatively linked to root elongation rate on the sugar beet genome (*RefBeet-1.1*).

Chromosome	Size [Mb]	Number of SNPs	Number of scaffolds
1	41.5	20	6
2	39.5	40	16
3	32.3	18	9
4	31.1	23	9
5	56.2	25	11
6	57.8	41	11
7	50.9	4	1
8	40.1	56	12
9	45.2	7	3

on chromosome 8 showed the highest number (13).

Single nucleotide polymorphisms were genotyped on the DNA of the individuals from the extremes of the phenotypic distribution for validation. A significant association was found for SNP10139 ($P < 0.01$). The sequences of the primers and the *TaqMan* probes designed for detection of SNP10139 are reported (Table 2 Suppl.). SNP10138 was the only SNP located on the same scaffold (Bvchr6.sca002) of SNP10139. Nevertheless, SNP10138 was statistically not associated to the RER trait ($P < 0.07$). The pattern of association between genotypes of SNP10139 and RER was also evaluated on 40 low and 40 high RER individuals of the F_2 population F290, showing a wide variation for the RER trait. Low and high RER samples were characterized by average RERs of 1.2 ± 0.02 and 2.8 ± 0.08 mm d^{-1}, respectively (Table 3). For this breeding population, SNP10139 also showed different allele frequencies between the two groups ($P < 0.01$).

On the sugar beet reference genome, the SNP10139 was mapped on chromosome 6 within the fourth exon of the *Bv6_128350_ktfi* gene (Fig. 2). Interestingly, Bv6_128350_ktfi sequence showed a high homology with the peptide transporter gene (*PTR*) family of *Arabidopsis thaliana* (Fig. 3). Among this family, *AtPTR2* (AT2G02040.1) showed the highest similarity (72.8 %) with the *Bv6_128350_ktfi* gene (Table 4). However, the SNP10139 is characterized by a mutation from A to G on the third base of the codon for leucine (UUA > UUG), which results in a silent mutation.

Table 3. Alleles of SNP10139 in low and high root elongation rate (RER) samples in the F290 F_2 population.

Low RER samples Sample ID	RER [mm d^{-1}]	SNP10139 genotype	High RER samples Sample ID	RER [mm d^{-1}]	SNP10139 genotype
L1	0.9	A/A	H1	2.4	G/A
L2	0.9	A/A	H2	2.4	A/A
L3	1.0	A/A	H3	2.4	A/A
L4	1.0	A/A	H4	2.4	G/G
L5	1.0	A/A	H5	2.4	A/A
L6	1.0	A/A	H6	2.5	G/G
L7	1.1	G/G	H7	2.5	G/G
L8	1.1	G/G	H8	2.5	G/A
L9	1.1	G/G	H9	2.5	G/G
L10	1.1	G/G	H10	2.5	A/A
L11	1.1	A/A	H11	2.6	G/G
L12	1.1	A/A	H12	2.6	G/G
L13	1.1	A/A	H13	2.6	G/A
L14	1.1	A/A	H14	2.6	G/A
L15	1.1	A/A	H15	2.6	A/A
L16	1.1	A/A	H16	2.6	A/A
L17	1.1	A/A	H17	2.7	G/G
L18	1.1	A/A	H18	2.7	G/G
L19	1.2	G/G	H19	2.7	A/A
L20	1.2	G/G	H20	2.7	A/A
L21	1.2	G/A	H21	2.7	G/G
L22	1.2	G/A	H22	2.7	G/A
L23	1.2	A/A	H23	2.7	G/A
L24	1.2	A/A	H24	2.7	A/A
L25	1.2	A/A	H25	2.7	A/A
L26	1.2	A/A	H26	2.7	A/A
L27	1.3	G/G	H27	2.7	A/A
L28	1.3	G/G	H28	2.8	G/G
L29	1.3	A/A	H29	2.8	G/G
L30	1.3	A/A	H30	2.8	G/A
L31	1.3	A/A	H31	2.8	G/A
L32	1.3	A/A	H32	2.8	A/A
L33	1.3	A/A	H33	2.9	A/A
L34	1.3	A/A	H34	2.9	A/A
L35	1.3	A/A	H35	3.1	G/G
L36	1.4	G/A	H36	3.2	A/A
L37	1.4	A/A	H37	3.4	G/A
L38	1.4	A/A	H38	3.8	G/G
L39	1.5	A/A	H39	3.8	G/G
L40	1.5	A/A	H40	5.1	G/A

Table 4. Amino acid sequence identities [%] among the peptide transporter genes *PTR* of *Beta vulgaris* (*Bv*) and *Arabidopsis thaliana* (*At*).

Species	Gene	No.	2	3	4	5	6	7
Bv	*Bv6_128350_ktfi*	1	72.8	62.0	56.1	60.4	59.1	41.8
At	*AtPTR2_AT2G02040.1*	2	-	68.0	62.7	59.1	57.1	40.2
At	*AtPTR6_AT1G62200.1*	3		-	63.1	53.2	52.8	37.8
At	*AtPTR4_AT2G02020.2*	4			-	47.2	45.9	35.9
At	*AtPTR1_AT3G54140.1*	5				-	73.9	42.3
At	*AtPTR5_AT5G01180.1*	6					-	41.6
At	*AtPTR3_AT5G46050.1*	7						-

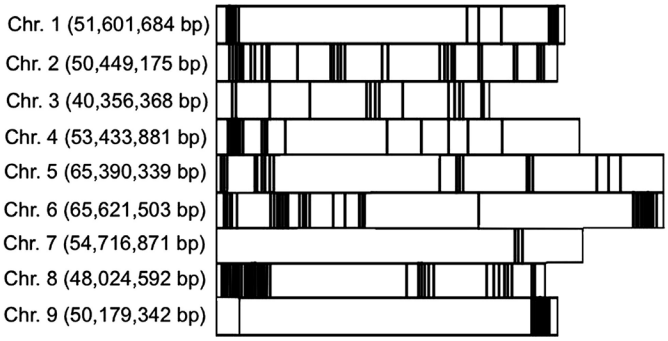

Fig. 2. Distribution of selected 234 single nucleotide polymorphisms across 9 sugar beet chromosomes (the *dark bars*).

Fig. 3. A schematic representation of the *Bv6_128350_ktfi* gene with the position of the single nucleotide polymorphism (SNP) 10139 according to the reference genome (*RefBeet-1.1*; http://bvseq.molgen.mpg.de).

Discussion

In this study, we demonstrated the feasibility of combining BSA and RAD approaches to generate a large number of candidate SNPs for association studies with RER in a format suitable for high-throughput genotyping. Our approach provides a good example of the high potential of RAD technology, combined with comparative assembly to the sugar beet genome, to characterize large numbers of informative SNPs in pooled DNA samples. Analogous approaches were successfully used to identify a panel of SNPs in eggplant (Barchi *et al*. 2011) and sugar beet (Stevanato *et al*. 2014).

Among associations between SNP mutations and the RER trait in sugar beet, we identified a strong association for SNP10139. Analogously, Rosas *et al*. (2013) found a SNP influencing root system architecture on two candidate genes (*RSA1* and *PHO1*) of *Arabidopsis thaliana*, and Kumar *et al*. (2014) revealed several SNPs within the *Rtcl*, *Rth3*, *Rum1*, and *Rul1* genes associated with seedling root traits in maize. The homologue peptide transporter gene (*PTR*) of Bv6_128350_ktfi, where SNP10139 was mapped, influences not only root development but also uptake of nitrate and peptides from the soil in *Arabidopsis thaliana* (Bai *et al*. 2013). Similarly, Komarova *et al*. (2008) showed that over-expression of a dipeptide transporter *AtPTR5* could enhance root growth and increase N content. Fan *et al*. (2014) demonstrated in rice that a di/tripeptide transporter *OsPTR6* increases both growth and N accumulation. These results could help to explain the previously found close association between morphological and related physiological root traits and productivity in sugar beet (Stevanato *et al*. 2010).

Intriguingly, SNP10139 is a silent mutation that does not result in amino acid change. A biological explanation for the effect of this SNP could be that it may be in linkage disequilibrium with another mutation in coding regions resulting in modification of expression of the gene. Alternatively, this SNP might change the substrate specificity of the RNA influencing the timing of translation and protein expression (Kimchi-Sarfaty *et al*. 2007). Numerous examples have been reported in the literature for linkages between silent mutations and

phenotype alterations (Goymer 2007, Garg *et al*. 2012, Jha *et al*. 2015).

Previous studies have demonstrated that differences in gene expression can be associated with quantitative traits and SNPs. Jaiswal *et al*. (2015) found a SNP modulating expression of the gene *TaGW2* associated with grain mass in wheat. Further studies will investigate both the functional effect of SNP10139 alleles on *PTR* gene expression and root morphology in sugar beet.

Root breeding has been proposed as a key factor for the "second green revolution" (Lynch 2007). Nevertheless, the contribution of sugar beet root traits as tools for selection of high yielding cultivars has not been adequately taken into account in breeding programs. The molecular marker associated with root growth identified here is one of the most efficient ways for improving root apparatus in sugar beet. The introgression of the SNP10139 allele into sugar beet genotypes might improve root soil exploration and nutrient acquisition. Previous studies in maize and sugar beet showed that a rapid primary root growth plays a major role in nutrient uptake and productivity, and it was hypothesized that alleles promoting root growth may facilitate selection for an efficient nutrient use (Vamerali *et al*. 2003, Stevanato *et al*. 2010).

In conclusion, this study provides a further indication of the polygenic control of root elongation rate in sugar beet. Moreover, our results suggest that the use of the SNP10139 marker in gene-assisted selection programs offers an opportunity to improve sugar beet root development and nutrient acquisition facilitating selection of high yielding cultivars.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J.: Basic local alignment search tool. - J. mol. Biol. **215**: 403-410, 1990.

Alvarez, J.M., Vidal, E.A., Gutierrez, R.A.: Integration of local and systemic signaling pathways for plant N responses. - Curr. Opin. Plant. Biol. **15**: 185-191, 2012.

Arnon, D.I., Hoagland, D.R.: Crop production in artificial culture solution and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. - Soil Sci. **50**: 463-483, 1940.

Bai, H., Euring, D., Volmer, K., Janz, D., Polle, A.: The nitrate transporter (NRT) gene family in poplar. - PLoS ONE **8**: e72126, 2013.

Barchi, L., Lanteri, S., Portis, E., Acquadro, A., Val, G., Toppino, L., Rotino, G.L.: Identification of SNP and SSR markers in eggplant using RAD tag sequencing. - BMC Genomics **12**: 304, 2011.

Cacco, G., Ferrari, G., Saccomani, M.: Pattern of sulfate uptake during root elongation in maize: its correlation with productivity. - Physiol. Plant. **48**: 375-378, 1980.

Cai, S., Bai, G.H., Zhang, D.: Quantitative trait loci for aluminum resistance in Chinese wheat landrace FSW. - Theor. appl. Genet. **117**: 49-56, 2008.

Conover, W.J.: Practical Non-parametric Statistics. - Wiley, New York 1980.

Courtois, B., Shen, L., Petalcorin, W., Carandang, S., Mauleon, R., Li, Z.: Locating QTLs controlling constitutive root traits in the rice population IAC 165 × Co39. - Euphytica **134**: 335-345, 2003.

De Dorlodot, S., Brian, F., Pages, L,, Price, A., Tuberosa, R., Xavier, D.: Root system architecture: opportunities and constraints for genetic improvement of crops. - Trends Plant Sci. **12**: 474-481, 2007.

Fan, X., Xie, D., Chen, J., Lu, H., Xu, Y., Ma, C., Xu, G.: Over-expression of *OsPTR6* in rice increased plant growth at different nitrogen supplies but decreased nitrogen use efficiency at high ammonium supply. - Plant Sci. **227**: 1-11, 2014.

Ganal, M.W., Altmann, T., Röder, M.S.: SNP identification in crop plants. - Curr. Opin. Plant Biol. **12**: 211-217, 2009.

Garg, B., Lata, C., Prasad, M.: A study of the role of gene *TaMYB2* and an associated SNP in dehydration tolerance in common wheat. - Mol. Biol. Rep. **39**: 10865-1086871, 2012.

Giuliani, S., Sanguineti, M.C., Tuberosa, R., Bellotti, M., Salvi, S., Landi, P.: *Root-ABA1*, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes. - J. exp. Bot. **56**: 3061-3070, 2005.

Goymer, P. Synonymous mutations break their silence. - Nat. Rev. Genet. **8**: 92, 2007.

Gupta, P.K., Rustgi, S., Mir, R.R.: Array-based high-throughput DNA markers for crop improvement. - Heredity **101**: 5-18, 2008.

Ho, C.H., Lin, S.H., Hu, H.C., Tsay, Y.F.: CHL1 functions as a nitrate sensor in plants. - Cell **138**: 1184-1194, 2009.

Jaiswal, V., Gahlaut, V., Mathur, S., Agarwal, P., Khandelwal, M.K., Khurana, J.P., Tyagi, A.K., Balyan, H.S., Gupta, P.K.: Identification of novel SNP in promoter sequence of TaGW2-6A associated with grain weight and other agronomic traits in wheat (*Triticum aestivum* L.). - PLoS ONE **10**:e0129400, 2015.

Jha, A.B., Tar'an, B., Diapari, M., Warkentin, T.D.: SNP variation within genes associated with amylose, total starch and crude protein concentration in field pea. - Euphytica **206**: 459-471, 2015.

Komarova, N.Y., Thor, K., Gubler, A., Meier, S., Dietrich, D., Weichert, A., Suter Grotemeyer, M., Tegeder, M., Rentsch, D.: *AtPTR1* and *AtPTR5* transport dipeptides in planta. - Plant Physiol. **148**: 856-869, 2008.

Kimchi-Sarfaty, C., OH, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V., Gottesman, M.M.: A "silent" polymorphism in the *MDR1* gene changes substrate specificity. - Science **315**: 525-528, 2007.

Kumar, B., Abdel-Ghani, A.H., Pace, J., Reyes-Matamoros, J., Hochholdinger F., Lybberstedt, T.: Association analysis of single nucleotide polymorphisms in candidate genes with root traits in maize (*Zea mays* L.) seedlings. - Plant Sci. **224**: 9-19 2014

Liang, Q., Cheng, X., Mei, M., Yan, X., Liao, H.: QTL analysis of root traits as related to phosphorus efficiency in soybean. - Ann. Bot. **106**: 223-234, 2010.

Lynch, J.P., Root architecture and plant productivity. - Plant Physiol. **109**: 7-13, 1995.

Lynch, J.P.: Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. - Ann. Bot. **112**: 347-357, 2013.

Lynch, J.P., Chimungu, J.G., Brown, K.M.: Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. - J. exp. Bot. **23**: 6155-6166, 2014.

Michelmore, R.W., Paran, I, Kesseli, R.V.: Identification of markers linked to disease-resistance genes by bulked segregant analysis: in specific genomic regions by using segregating populations. - Proc. nat. Acad. Sci. USA **88**: 9828-9832, 1991.

Nakamura, T., Adu-Gyamfi, J.J., Yamamoto, A., Ishikawa, S., Nakano, H., Ito, O.: Varietal differences in root growth as related to nitrogen uptake by sorghum plants in low-nitrogen environment. - Plant Soil **245**: 17-24, 2002.

Nazoa, P., Vidmar, J.J., Tranbarger, T.J., Mouline, K., Damiani, I., Tillard, P., Zhuo, D., Glass, A.D., Touraine, B.: Regulation of the nitrate transporter gene *AtNRT2.1* in *Arabidopsis thaliana*: responses to nitrate, amino acids and developmental stage. - Plant mol. Biol. **52**: 689-703, 2003.

Nour-Eldin, H.H., Andersen, T.G., Burow, M., Madsen, S.R., Jorgensen, M.E., Olsen, C.E., Dreyer, I., Hedrich, R., Geiger, D., Halkier, B.A.: NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. - Nature **488**: 531-534, 2012.

Pegadaraju, V., Nipper, R., Hulke, B., Qi, L., Schultz, Q.: *De novo* sequencing of sunflower genome for SNP discovery using RAD (Restriction site Associated DNA) approach. - BMC Genomics **14**: 556, 2013.

Price, A.H., Cairns, J.E., Horton, P., Jones, H.G., Griffiths, H.: Linking drought resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. - J. exp. Bot. **53**: 989-1004, 2002.

Raman, H., Moroni, J.S., Sato, K., Read, B.J., Scott, B.J.: Identification of AFLP and microsatellite markers linked with an aluminum tolerance gene in barley (*Hordeum vulgare* L.). - Theor. appl. Genet. **105**: 458-464, 2002.

Rosas, U., Cibrian-Jaramillo, A., Ristova, D., Banta, J.A., Gifford, M.L., Fan, A.H., Zhou, R.W., Kim, G.J., Krouk, G., Birnbaum, K.D., Purugganan, M.D., Coruzzi, G.M.: Integration of responses within and across *Arabidopsis* natural accessions uncovers loci controlling root systems architecture. - Proc. nat. Acad. Sci. USA **110**: 15133-15138, 2013.

Saccomani, M., Cacco, G., Ferrari, G.: Efficiency of the first steps of sulfate utilization by maize hybrids in relation to their productivity. - Physiol. Plant. **53**: 101-104, 1981.

Saengwilai, P., Tian, X., Lynch, J.P.: Low crown root number enhances nitrogen acquisition from low-nitrogen soils in maize. - Plant Physiol. **166**: 581-589, 2014.

Salmenkallio, M., Sopanen, T.: Amino acid and peptide uptake in the scutella of germinating grains of barley, wheat, rice, and maize. - Plant Physiol. **89**: 1285-1291, 1989.

Sanguineti, M.C., Giuliani, M.M., Govi, G., Tuberosa, R., Landi, P.: Root and shoot traits of maize inbred lines grown in the field and in hydroponic culture and their relationships with root lodging. - Maydica **43**: 211-216, 1998.

Seeb, J.E., Carvalho, G., Hauser, L., Naish, K., Roberts, S., Seeb, L.W.: Single nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. - Mol. Ecol. Res. **11**: 1-8, 2011.

Stevanato, P., Broccanello, C., Biscarini, F., Del Corvo, M., Sablok, G., Panella, L., Stella, A., Concheri, G.: High-throughput RAD-SNP genotyping for characterization of sugar beet genotypes. - Plant mol. Biol. Rep. **32**: 691-696, 2014.

Stevanato, P., Trebbi, D., Saccomani, M.: Root traits and yield in sugar beet: identification of AFLP markers associated with root elongation rate. - Euphytica **173**: 289-298, 2010.

Tegeder, M., Rentsch, D.: Uptake and partitioning of amino acids and peptides. - Mol. Plant. **3**: 997-1011, 2010.

Tnani, H., Lopez-Ribera, I., Garcia-Muniz, N., Vicient, C.M.: ZmPTR1, a maize peptide transporter expressed in the epithelial cells of the scutellum during germination. - Plant Sci. doi: 10.1016/j.plantsci.2013.03.005, 2013.

Tuberosa, R., Sanguineti, M.C., Landi, P., Giuliani, M.M., Salvi, S., Conti, S.: Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. - Plant mol. Biol. **48**: 697-712, 2002.

Vamerali, T., Saccomani, M., Bona, S., Mosca, G., Guarise, M., Ganis, A.: A comparison of root characteristics inrelation to nutrient and water stress in two maize hybrids. - Plant Soil **255**: 157-167, 2003.

Wang, H., Taketa, S., Miyao, A., Hirochika, H., Ichii, M.: Isolation of a novel lateral-rootless mutant in rice (*Oryza sativa* L.) with reduced sensitivity to auxin. - Plant Sci. **170**: 70-77, 2006.