Utilization of *M. caerulea* gene pool in alfalfa breeding for acid tolerance

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In Serbia it is of great importance to broaden growing area of alfalfa on light or medium acid soils, since it coincides with main livestock production regions. Alfalfa breeding focused on conventional methods did not succeed to improve genetic base of tolerance to soil acidity, or these results are very modest. The lack of Al-tolerant alfalfa cultivars can be attributed to multiple factors including the absence of protocols for evaluating and selecting plant response to Al. Main objective of this paper is to establish breeding strategy for acid tolerance in alfalfa using *M. caerulea* gene pool. For this work we used diploid population of *M. caerulea* - PI 464724 which carries AL tolerance according to previous researches. Population PI 464724 was sown in field in April 2012 (25 plants). They were put in open pollination, surrounded by tetraploid sativa genotypes. Idea was to utilize in field conditions production of 2n unreduced gametes in diploid *M. caerulea* and to favor 2x - 4x crosses in field. Only one plant survived and produced pods, which were harvested in August 2012. Seed was sown in green house in January of 2013. Differences in seed size were recorded, which could have originated from different ploidy levels of seed. All seeds were sawn in the pots, in the greenhouse but only 11 plants fully developed. During the vegetation distinction between plants was detected: 10 plants looked as tetraploid genotypes, while 1 plant looked as tipical *M. caerulea*. Initial ploidy-level classification was based on morphology of the plants in greenhouse. Using this procedure 1 plant with small leaves and thin stems was classified as diploid. Between 10 plants which resembling tetraploid genotypes differences were also recorded, 5 plants looked as tipical sativa genotypes, and other 5 were more similar to diplod *M. caerulea* plants. Putative tetraploids (5 plants) with large green leaves were crossed by hand to produce progenies. Ploidy level of tested plants was analysed by using cytological analysis and multiplication with SSR markers. Cytogenetic analysis showed that all 11 plants were fertile. Pollen viability ranged from 21.45 to 97.09%, and the average number of ovules per plant ranged from 8.80 to 12.29. Measured size of pollen grains indicates the possible existence of two ploidy levels. Pollen grains were bigger at six plants (38.43/34.75μm to 41.01/37.04μm), and smaller at 5 plants (33.64/31.81μm to 25.69/33.20μm). After amplification with 23 SSR markers, one plant had only two alleles detected for all primer pairs. The three or four alleles were detected in all other plants after multiplications with at least one primer. For further investigation seed from crosses was taken from plants which were recorded as tetraploids using both morphological and molecular markers (5 parental plants). Next steps: progenies from crosses of tetraploid plants (200 plants), together with *M. sativa* ssp. *sativa* Serbian cultivar Niagara (200 plants) and PI 464724 (200 plants) as controls will be planted in field in southwest Serbia in soil with pH level $\leq$5.0 in spring of 2014. In the field conditions the genotypes will be tested for Al tolerance Next growing season (2015), surviving *M. caerulea* progenies will be multiplied in open pollination in order to produce F_2 plants. This F_2 progenies will be tested for Al tolerance and agronomic performance in field conditions at the same site. Al tolerant genotypes with desirable agronomic traits (yield, quality, persistence), will be planted at experimental field, in Novi Sad, and backcrossed in few generation with tetraploid *M. sativa* lines designated for growing on soils with poor performance. Their progenies will be multiplied in the field for development of Al tolerant alfalfa germplasm. This type of research represent first attempt in Serbia related to hybridization between *M. sativa* and *M. caerulea* in order to obtain tetraploid gemrplasm tolerant to soil acidity. Results obtained in field condition of Serbia together with strategies based on conventional and molecular marker breeding efforts, could lead toward development of tolerant alfalfa cultivars and successful production of this crop on acid, and Al toxic soils.