A response to nitrogen shortages in recombinant *afila* lines of the mapping population of field pea (*Pisum sativum* L.).

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Introduction.
In European countries with the predominant podzolic soils, productivity of field pea is affected by instabilities in water and mineral accessibility. In the stress conditions, yield potential and qualitative seed properties depend on plant physiological capacities to an efficient utilization of environmental resources (water, minerals, CO_2). Hence, the pro-ecological strategy to breed efficient cultivars better adapted to less favourable environments is justified and could stabilize pea production.

The present project was initiated to identify pea genome regions responsible for the physiological components of water and nitrogen economy. In the study done under controlled and field conditions, the components were examined among parents and chosen lines from the Canadian mapping population [Carneval × MP1401] during the whole growth season under varied nitrogen nutrition.

Results and Discussion
Eighteen recombinant lines from the mapping pea population [Carneval × MP1401] were chosen based on field yield in 2013 (9 lines with the lowest and 9 with the highest yield). These RILs and the parental lines were examined in the controlled greenhouse and in field conditions under varied nutrients supply. Nitrogen use parameters like **Gw/Ng** and **$NUtE_{veg}$** were higher in field conditions than in the greenhouse. Nitrogen use effciency in seed formation **$NUtE_{gen}$** and **NHI** were lower in field conditions than in the greenhouse (Tab. 2). All characters were significantly affected by soil treatments. Generally, yield characteristics and the season-long TE efficiencies decreased under nitrogen-limited conditions (Tab.3). Genotype-treatment (G-E) interaction effects were significant for most characters at the final growth phase. Noteworthy, relationships between yield and water and nitrogen efficiency were stronger in nitrogen-limited conditions suggesting an enhanced importance of the physiological components for pea yielding under sub-optimal conditions.
The parental line MP1401 had the higher tolerance index **T** for the nitrogen shortage than the line Carneval (1,02 versus 0,68) in the controlled greenhouse. The line MP1401 was more tolerant to nitrogen shortage because of higher **N_{fix}** than in the line Carneval (N_{fix} 61% versus N_{fix} 54%). The MP1401 was more stable in yielding than the line Carneval in stress (5,75 g/ plant in the control conditions and 5,4 g/ plant in the nitrogen shortage for MP1401 versus 5,8 g/ plant in the control conditions and 3,7 g/ plant in the nitrogen shortage for Carneval).

Variability source	Yield				Water use	
	Yield		HI		$WUtE_{GEN}$	$WUtE_{VEG}$
	field	pots	field	pots	pots	pots
	g dm/ plant	g dm/ plant	%	%	mg mol^{-1}	mg mol^{-1}
Nitrogen level						
N optimum	5,1	5,5	43	13,8	34,1	31,4
Low N	3,7	5,1	41	5,2	28,0	25,2
N optimum, Przebędowo	3,6		34			
ANOVA						
Genotypes	*	**	**	**	**	**
N fertilization level	**	*	**	ns	**	**
G x Treat	*	+	**	ns	ns	*

+, *, ** - significant P<0.10, P<0.05 and P<0.01, respectively

Table 3. The yield and WUE under the nitrogen optimum and the N shortage conditions.

Sample	Macroelements							pH
	mg·100 g^{-1} d.m. soil							(H_2O)
	N-NH_4	N-NO_3	P	K	Ca	Mg	S-SO_4	
1. Wiatrowo, low N,	**0,4**	traces	4,1	7,9	26,9	4,3	**0,1**	6,37
2. Wiatrowo N optimum (after suppl. **1,4 mg N**)	**0,4**	**0,4**	**6,9**	**11,0**	**45,9**	**7,3**	**0,1**	**6,57**
3. Przebędowo, stress field (**1,5 mg N**)	**0,4**	**1,1**	4,3	**17,1**	**117,2**	**11,2**	**0,2**	**7,27**
Indicator content	N-NH_4+N-NO_3 2,5-5,0		3,0-6,0	5,0-8,0	25,0-40,0	3,0-6,0	1,0-3,0	5,5-6,5
	mg/ dm^3 soil							
Pots, High N	**100**							
Pots, Low N	**43**							

Table 1. The soil analysis for N experiments.

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Variability source	Nitrogen assimilation efficiency		Nitrogen use efficiency							
	NUpE	Nfix	NHI		Gw/Ng		$NUtE_{GEN}$		$NUtE_{VEG}$	
	pots	pots	field	pots	field	pots	field	pots	field	pots
	(%)	(%)	(%)	(%)	(mg mg-1)	(mg mg-1)	(g g-1)	(g g-1)	(g g-1)	
Nitrogen level										
N optimum	155	35	77	85	29,5	27,8	22,6	23,5	30,1	19,4
Low N	411	74	73	83	30,7	24,7	22,3	20,5	32,8	17,4
N optimum, Przebędowo			64		26,9		17,3		35,5	
$Lsd_{0,05}$	24	4		2		1,1		1,2		1,0
ANOVA										
Genotypes	**	**	**	*	**	**	+	**	*	**
N fertilization level	**	**	**	ns	*	**	**	**	**	**
G x Treat	**	**	*		ns	**	*	ns	*	**

+, *, ** - significant P<0.10, P<0.05 and P<0.01, respectively

Table 2. Components of N efficiency under optimal and the reduced N supply.

Figure 1. [Carneval × MP1401] RILs in the controlled greenhouse and in field conditions under varied nitrogen supply.

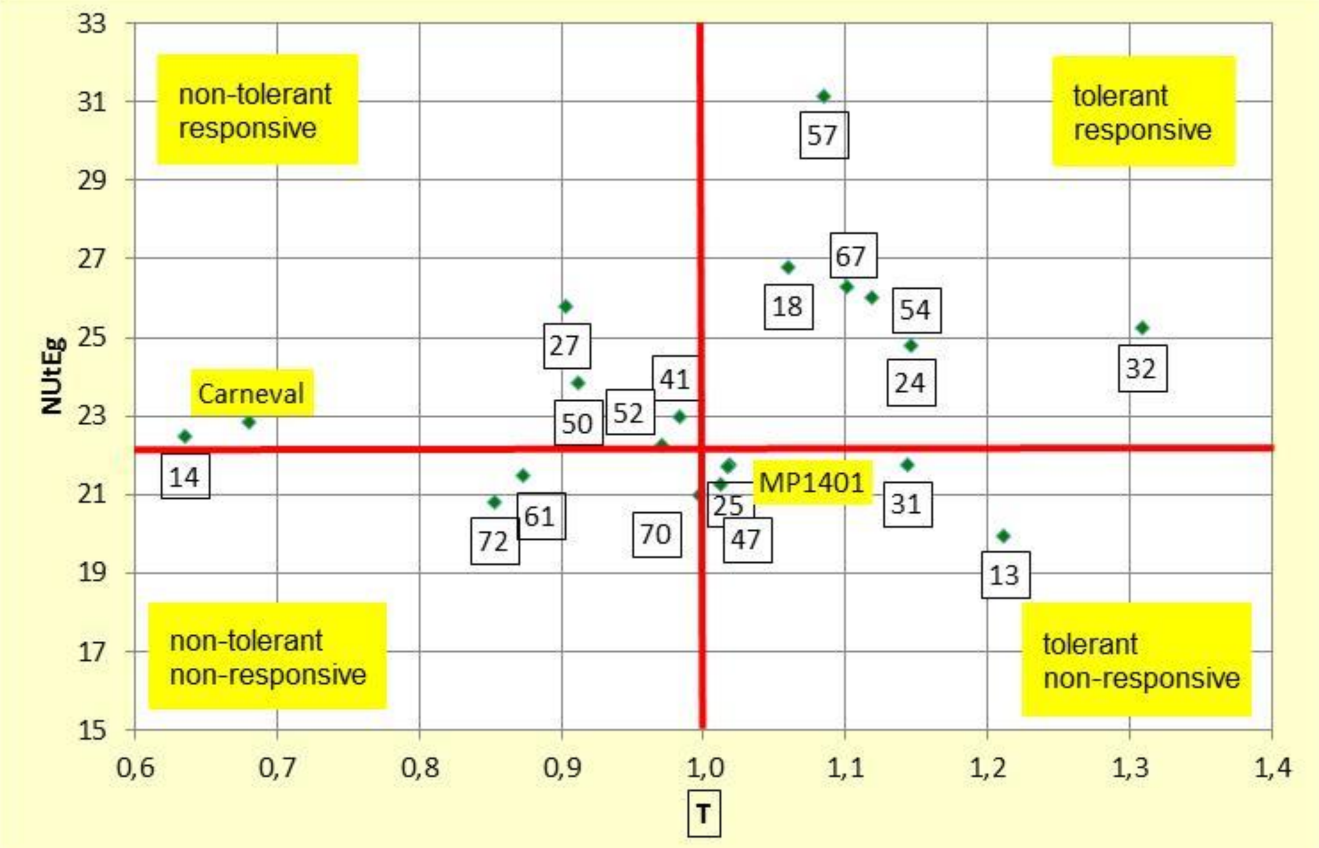

Figure 2. Categorization of the parents and the RIL lines for the yield-indicated tolerance to low N and NUtE-response index (g g^{-1}), i.e. a physiological ability to utilize each additional N unit with an increased efficiency.

Materials and methods.
The material consists of parental lines and 18 chosen recombinant lines from the mapping pea population [Carneval × MP1401] with *afila* leaves (Tar'an et al. 2003). The materials were grown in soil pots in a controlled greenhouse and in the field conditions under varied nutrients supply (Tab.1). The amount of transpired water (WT) during the growth season was determined using the gravimetric method. In season-long scale, the transpiration efficiency was estimated as plant dry weight/ WT ratio. In addition, N uptake and other components of N utilization efficiency were determined.
Experiment 1
- partially controlled greenhouse; 8-30/6-16°C day/night, photoperiod 10-17h, 400-1600 μM m^{-2} s^{-1} PAR at the plant level, 40-85% RH (depending upon the growth phase and photoperiod cycling);
- double-sided Kick-Brauckman's pots (9 dm^3 of soil) with a surface perlite layer;
- two soil treatments: control (relatively high N, 105 mgN/dm^3) and low N (42 mg/dm^3);
- soil water content maintained constant (70-75% FWC); amounts of water transpired recorded by frequent weighing of pots during the whole growth season;
- yield traits and water use measurements (done until the full maturity). Whole plants were hand-harvested at full maturity. The vegetative (stems + leaves) and generative (seeds) plant parts were separated and their dry weights (g d.w. pot^{-1}) were determined by oven drying at 65°C for 72 h. Nitrogen concentrations (**seed N%** and **straw N%**) in the dried and ground homogeneous sub-samples of the parts (IKA mill processed) were measured using high-temperature combustion (Dumas' method) in the elemental analyser (VarioMax-CN, Elementar Ltd., Germany). Using the seed and straw N concentrations, **nitrogen uptake** (**NUp**, g m^{-2} or g pot^{-1}), i.e. total amounts of N taken up by plants as a sum of N accumulated in vegetative parts and seeds, were estimated. According to Moll et al. (1982), the following components of N efficiency were determined: **NUE** (%), N uptake efficiency; **NHI** (%), N harvest index; **Gw/Ng** (mg mg^{-1}); seed dry weight produced per unit of N accumulated in seeds, **NUtE** (g g^{-1}); **Gw/NUp ratio**, i.e. physiological index of the efficiency of N utilization in seed mass formation (syn. N utilization efficiency ratio). Tolerance index (**T)** was determined for each i-th entry on the basis of its grain yield (d.w. m^{-2}) using a modification of the standard equation (Fischer and Maurer 1978): $T = (L_i/H_i)/D$, where L_i and H_i are the grain yields of the i-th entry under low and high N nutrition, respectively, while D-value (=overall mean L/overall mean H) is a general measure of stress intensity in the experiment.

The plant ability to respond to variable amounts of nitrogen with an increased efficiency of utilization in grain mass formation is an important component of plant adaptation to gradients of nutrient supply (Górny *et al.* 2011). The analysed lines were categorized for tolerance for low N conditions and physiological ability to utilize absorbed N efficiently. Six lines had enhanced tolerance to low N and exhibited the greatest ability to utilize additional N with an enhanced efficiency; these genotypes can produce well under low N fertilization and are able to respond well with enhanced N efficiency (tolerant and responsive group, Fig.2). Four tolerant lines with parental line MP1401 had limited physiological ability to utilize additional N efficiently and this group was described as a tolerant and non-responsive (Fig.2). Five genotypes and the parental line Carneval with a limited yield potential under low N and a reduced ability to utilize additional N efficiently were classified in non-tolerant and responsive group (Fig.2). Three lines were in the non-tolerant and non-responsive group (Fig.2). According to Fageria and Baligar (1997), lines belonging to the 1st group (above all) and 2nd group (to a lesser extent) appear to be the most desirable materials for breeding programs that deal with adaptation to low-input agriculture. Results of the study show that N fertilization levels have a critical influence on the expression of gene actions governing N efficiency in pea. The effects of genotype × environment interaction for components of the efficiency suggest that a genotype most efficient in N uptake and N utilization under optimal conditions is not necessarily the most efficient under limited N.

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