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In vitro method for early evaluation of nitrogen use efficiency associated traits in potato

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Summary

The objective of the present study was to characterize various traits associated with nitrogen uptake and utilization in a range of potato cultivars. For this purpose an *in vitro* test system was developed which allows analyzing specific stress responses in a highly controlled environment. Shoot tips were grown fixed in perforated stainless steel plates in 500 ml glass vessels in liquid culture medium at four nitrogen levels, i.e. 60, 30, 15 and 7.5 mmol L^{-1}. At the end of a three weeks' culture period plant developmental traits were determined and nitrogen uptake and assimilation were analyzed. Reduction of nitrogen in the culture medium differentially affected morphological and physiological features. Highly significant differences were found between different N-levels and cultivars as well as for genotype x nitrogen level interactions. Three groups of cultivars (high, low and intermediate) were distinguished with respect to biomass production and crude protein yield under nitrogen-sufficient conditions of 60 mmol L^{-1}. Genotypes with a low biomass production at full nitrogen availability responded with increased root development under nitrogen deficiency stress and increased their nitrogen utilization capacity in relation to the other cultivars.

Introduction

In recent years, the need for a sustainable exploitation of resources has attained increased attention in agricultural production. Nitrogen fertilization is a key element for obtaining profitable yields but, at the same time, it implies the risk of environmental pollution. Only a limited proportion of the applied nitrogen is actually taken up by plants, while as much as 60 to 70 % are lost from the plant-soil system by gaseous emissions due to soil denitrification and ammonia volatilisation as well as leaching of nitrate (e.g. Raun and Johnson, 1999; Good et al., 2004; Vos, 2009). Resulting adverse environmental effects include pollution of ground-, fresh- and marine water as well as the release of greenhouse gases as N_2O with its high impact on global warming. Two strategies have been pursued in order to minimize the negative impact of nitrogen fertilizers on the environment. One approach is the development of enhanced-efficiency fertilizers and optimized nutrient management (Hopkins et al., 2008), while the second focuses on selection of cultivars with improved nitrogen use efficiency (NUE). In this context, efficient methods for screening of germplasm are needed.

Commercial potato production is especially prone to lead to environmental contamination, as cultivation on sandy soils in combination with high nitrogen fertilizer rates and irrigation results in leaching of nitrate (Sharifi et al., 2007). Furthermore, the shallow root system of *Solanum tuberosum* does not allow nitrogen retraction from deeper soil layers. A survey on nitrate-N contaminations of rural well water in agricultural regions of Canada demonstrated that the mean NO_3-N concentration was in fact associated with the proportion of the area cropped to potatoes (Demerchant et al., 1990).

Nutrient use efficiency is regarded as the second most important goal next to drought tolerance in abiotic stress improvement of crops (Hirel et al., 2011). Many attempts have been made in order to elucidate the genetic and physiological basis of this complex trait (reviewed for example by Hirel et al., 2007; Hirel et al., 2011; Lea and Azevedo, 2006; Lea and Azevedo, 2007; Lightfoot, 2010). However, the current knowledge of key steps involved in the control of nutrient use efficiency from gene expression to metabolic activity and phenotypic manifestation remains incomplete (Hirel et al., 2011). In spite of various experiments which have been performed with potato worldwide to identify genotypic differences in NUE from an agronomic point of view, information on underlying physiological mechanisms of nutrient uptake and utilization efficiency is limited (Sharifi et al., 2007).

The objective of the present investigation was to characterize various traits associated with nitrogen uptake and utilization in a range of potato cultivars at an early growth stage in a highly controlled environment in order to get detailed knowledge on genetic differences in N use efficiency. For this purpose an *in vitro* test system was developed with the aim to possibly identify specific morphological and physiological components which are crucial for nitrogen use efficiency in *Solanum tuberosum* L.

Materials and methods

Plant material

Thirteen mid-early potato cultivars (maturity group III) developed by different breeding companies were used for this investigation, i.e. 'Afra', 'Agria', 'Ditta', 'Filea', 'Marlen', 'Nicola', 'Simone', 'Solara', 'Topas' (Europlant Pflanzenzucht, Germany) 'Pirol', 'Lambada', (Norika, Germany), 'Skala' (Bavaria-Saat, Germany) as well as 'Milva' (DNK 1970; an old Danish accession derived from the IPK (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany). Stock cultures of virus tested *in vitro* plantlets were kept in 250 ml glass vessels on gelrite solidified MS medium (Murashige and Skoog, 1962) without growth regulators. Cultures were incubated at 18 °C in a 16-h-photoperiod and subcultured every four to six weeks. 14 days before initiation of the experiments 10 nodal sections were placed in each 250 ml culture vessel and incubated as stated above. Shoot tips of approximately 1.5 - 2.0 cm length were excised from the newly developed sprouts and used for nitrogen stress experiments.

In vitro test system

For the experiments 10 shoot tips were fixed in perforated stainless steel plates and incubated in 500 ml glass vessel containing 50 ml liquid culture medium. The nitrogen levels of the media corresponded to full, 1/2, 1/4 and 1/8 of the original MS concentration (60, 30, 15, 7.5 mmol L^{-1}), providing NH_4^+-N and NO_3^--N in a ratio of 0.52. Cultures were incubated at 18 °C in a 16-h-photoperiod for a period of 21 days. At that time, specific morphological traits of each individual plantlet were recorded, i.e. shoot length, number of nodes for calculating internode length, and the chlorophyll content of the youngest fully expanded leaf measured with a SPAD-502 chlorophyll meter (Konica Minolta). In addition, fresh and dry matter of both

shoots and roots were determined for each unit of 10 plantlets. The remaining nutrient solutions were frozen at -20 °C until analysis of the nitrogen withdrawal. Assays were repeated in three independent time-shifted experiments each time comprising four replicates per N-variant.

Analysis of nitrate, ammonium and crude protein

The remaining nitrogen in media at the end of the culture period was determined spectrophotometrically as described by SCHUM and JANSEN (2012). The quantity of nitrate-N was roughly determined by use of MQuant™ test strips (Merck KGaA) and solutions were diluted as appropriate. Final analysis was performed by application of a spectrophotometric method adapted from the official nitrate analysing method of soil samples of the German Association of Agricultural Experiment and Research Stations (VDLUFA, 2002) which determines the nitrate-N content by measuring the difference in UV-absorption at 210 nm of the original solution and after nitrate reduction by nascent hydrogen. Ammonium-N was determined spectrophotometrically according to DIN 38406-5 of German standard methods for the examination of water, waste water and sludge (Normungsausschuss Wasserwesen 1983), in which NH_4^+-ions react at a high pH-value with hypochlorite- und salicylate-ions in the presence of sodium nitroprusside to form the salicylic acid analog of indophenols. The lowest concentration used for calibration was 0.5 mg/l in case of nitrate-N and 0.25 mg/l in case of ammonium-N, respectively.

Crude protein contents of shoots and roots were analyzed by Near Infrared Reflectance Spectroscopy (NIR) based upon calibration by standard Kjeldahl methods (SCHUM and JANSEN 2012). Shoots and roots of plantlets cultured for three weeks on media with four different nitrogen levels were dried at 60 °C for 24 hours. Dry matter was milled in a Retsch laboratory swing mill and stored in Eppendorf tubes. Upon calibration by application of the standard Kjeldahl methods, crude protein content of samples were determined by Near Infrared Reflectance Spectroscopy (NIR). Reflexion spectra were measured with a Multi Purpose Analyzer (BRUKER OPTICS) using a wavelength range between 800 and 2500 nm. The device was equipped with a small flat-bottomed glass cuvette to take up the milled plant material. 150 samples of shoots and 202 samples of roots were selected in order to correlate the individual spectra with the protein amount of specific probes as determined by the Kjeldahl method. The calibration provided results for predicting the protein content with $r^2 = 0.991$ (root mean standard error of prediction RMSEP = 0.85) for shoots and $r^2 = 0.979$ (RMSEP = 0.77) for roots, respectively. The minimum amount of determinable crude protein was limited by the amount of dry matter produced by the plantlets. At least 10 mg were necessary to cover the bottom of the cuvette. The lowest amounts determined were found in roots of plantlets grown in media with 1/8 N-supply with 6.3 % of dry matter. As the routine Kjeldahl process does not determine nitrate and nitrite nitrogen, solely nitrogen metabolized by the plantlets into organic compounds was evaluated.

Stress susceptibility index

Ranking of cultivars was performed for selected features by computing the stress susceptibility index (SSI) introduced by FISCHER and MAURER (1978) according to following formula:

$$SSI = (1-Ps / Pc) / (1- meanPs / meanPc)$$

Ps = parameter determined under stress conditions (1/2N)
Pc = parameter determined under control conditions (1N)
meanPs = mean of all genotypes under stress conditions
meanPc = mean of all genotypes under control conditions

Statistical analysis

Data collected from the three independent time-shifted experiments with four replicates of each variant were subjected to analysis of variance by application of the general linear model (GLM) procedure of SAS 9.2.

Results

Plant performance

Reduction of nitrogen in the culture medium differentially affected a wide range of morphological and physiological features in a genotype specific manner. Highly significant differences were found between N-levels and cultivars as well as for genotype x nitrogen level interactions (Tab. 1).

The stepwise decrease in nitrogen supply from the 1/2 to the 1/4 and 1/8 N level is clearly reflected by a reduction in shoot and internode length, chlorophyll content of the youngest fully developed leaf as well as in fresh matter production of shoots, roots and whole plantlets (Tab. 1). However, the degree of impairment caused by nitrogen deficiency stress depends on the genotype. This is indicated in Tab. 1 by highlighting thresholds for a significant decline in trait performance for individual cultivars. The analysis of variance revealed significant differences between genotypes e.g. in fresh matter yield of plantlets at each individual nitrogen level as well as differential responses of individual genotypes to the reduction of available nitrogen. While cultivars 'Lambada', 'Milva' and 'Solara' produced the highest amount of plant biomass under full nitrogen supply, these genotypes showed a strong and continuous decline with the reduction of nitrogen availability immediately beginning at the 1/2 N-level. In contrast, for the majority of genotypes a significant drop in fresh matter production was observed only upon further reduction of the nitrogen availability to 1/4. The two cultivars with low biomass production at a high nitrogen level ('Agria' and 'Topas') showed a better performance under deficiency stress conditions and fell off only at the 1/8 N level. Fresh matter of cv. 'Topas' produced at 1/4 and 1/8 of the original nitrogen concentration reached values comparable to those of the normally vigorously growing genotype 'Lambada'.

The discrimination between plant parts revealed that differences in biomass production in response to nitrogen deficiency stress were generally more pronounced for roots as for shoots. The root percentage share of total biomass varies between 22 % ('Filea') and 59 % ('Lambada') at full nitrogen supply. Upon reduction of available nitrogen two fundamentally different types of response could be distinguished between genotypes after three weeks of culture as demonstrated in Fig. 1. In some cultivars root fresh matter continually decreased with increasing nitrogen deficiency stress. In contrast, other cultivars responded with a stimulation of root development upon reduction of the nitrogen supply to 1/2 of the original MS medium and were even able to sustain root proliferation at the 1/4 level. It has to be noted that in spite of the general reduction of root proliferation with decreasing nitrogen availability in 'Lambada', this cultivar still produced the highest amount of root biomass at all N-levels as compared to the rest of the genotypes. In contrast, absolute values for root fresh matter of 'Topas' increased under nitrogen deficiency stress in relation to the other cultivars.

Further analysis of biomass production in relation to nitrogen supply was based on the determined dry matter. In order to visualize type and dimension of modifications of single characters in response to nitrogen deficiency stress we followed the procedure used by IKRAM et al. (2012). For each evaluated trait mean values determined under nitrogen deficiency stress (1/2 N, 1/4 N, 1/8 N) were plotted on the vertical axis against values obtained at full nitrogen availability on the horizontal axis. This approach allows compiling of data from 13 genotypes and 4 nitrogen levels within one graph. Further

Tab. 1: Vegetative development of 13 potato genotypes in response to increasing nitrogen deficiency stress. Differences between N-levels, genotypes and corresponding interactions are illustrated in terms of F-values. In addition, means for single traits are shown for individual cultivars and N-levels. (Af = 'Afra'; Ag = 'Agria'; Di = 'Ditta'; Fi = 'Filea'; La = 'Lambada'; Ma = 'Marlen'; Mi = 'Milva'; Ni = 'Nicola'; Pi = 'Pirol'; Si = 'Simone'; Sk = 'Skala'; So = 'Solara'; To = 'Topas')
(Values for individual genotypes marked with different letters are significantly different according to LSD based multiple comparison test [p=0.05]. Thresholds for a significant decline in performance are highlighted.)

Trait	**F-Values**			**N**	**Plant Performance with Decreasing N Supply for Individual Traits and Genotypes**												
	N-Level (N)	**Geno-type(G)**	**N x G**	**Level**	**Ag**	**To**	**Ni**	**Fi**	**Ma**	**Di**	**Pi**	**Sk**	**Si**	**Af**	**So**	**Mi**	**La**
FM Plants [g per 10]	801.70***	51.73***	7.43***	1N	5.84^{a}	6.05^{a}	7.20^{a}	6.18^{a}	6.68^{a}	6.83^{a}	7.58^{a}	7.81^{a}	7.98^{a}	9.53^{a}	9.30^{a}	11.11^{a}	11.16^{a}
				1/2N	6.83^{b}	7.36^{b}	7.71^{a}	6.43^{a}	6.22^{a}	7.16^{a}	7.69^{a}	7.46^{a}	7.96^{a}	8.98^{a}	7.80^{b}	9.67^{b}	8.83^{b}
				1/4N	5.40^{a}	6.13^{a}	5.62^{b}	4.21^{b}	4.57^{b}	4.68^{b}	5.43^{b}	5.68^{b}	5.95^{b}	5.82^{b}	5.05^{c}	6.89^{c}	6.04^{c}
				1/8N	3.29^{c}	3.87^{c}	3.72^{c}	2.48^{c}	3.03^{c}	2.90^{c}	3.28^{c}	3.67^{c}	3.78^{c}	3.96^{c}	3.20^{d}	4.73^{d}	4.00^{d}
FM Shoots [g per 10 plants]	667.14***	26.54***	3.62***	1N	4.01^{a}	3.77^{a}	5.04^{a}	4.80^{a}	4.63^{a}	4.16^{a}	4.72^{a}	4.51^{a}	4.23^{a}	5.56^{a}	6.05^{a}	6.46^{a}	4.61^{a}
				1/2N	4.52^{b}	4.67^{b}	4.61^{a}	4.18^{b}	4.23^{a}	4.53^{b}	4.72^{a}	4.55^{a}	4.38^{a}	5.09^{a}	4.93^{b}	5.83^{b}	4.33^{a}
				1/4N	3.26^{c}	3.61^{a}	3.21^{b}	2.76^{c}	3.20^{b}	3.08^{c}	3.36^{b}	3.56^{b}	3.28^{b}	3.58^{b}	3.56^{c}	4.32^{c}	2.99^{b}
				1/8N	2.11^{d}	2.25^{c}	2.30^{c}	1.74^{d}	2.12^{c}	1.96^{d}	2.02^{c}	2.38^{c}	2.32^{c}	2.57^{c}	2.34^{d}	3.02^{d}	2.10^{c}
FM Roots [g per 10 plants]	280.91***	56.23***	8.89***	1N	1.83^{a}	2.28^{a}	2.05^{a}	1.38^{a}	2.12^{a}	2.67^{a}	2.86^{a}	3.30^{a}	3.75^{a}	3.98^{a}	3.14^{a}	4.65^{a}	6.55^{a}
				1/2N	2.32^{b}	2.70^{a}	3.10^{b}	2.30^{b}	1.99^{a}	2.63^{a}	2.97^{a}	2.91^{a}	3.58^{a}	3.89^{a}	2.82^{a}	3.90^{b}	4.50^{b}
				1/4N	2.14^{ab}	2.51^{a}	2.41^{a}	1.46^{a}	1.37^{b}	1.60^{b}	2.07^{b}	2.11^{b}	2.68^{b}	2.24^{b}	1.50^{b}	2.57^{c}	3.05^{c}
				1/8N	1.19^{c}	1.62^{b}	1.42^{c}	0.74^{c}	0.91^{c}	0.94^{c}	1.26^{c}	1.29^{c}	1.46^{c}	1.39^{c}	0.87^{c}	1.71^{d}	1.90^{d}
Shoot Length [cm]	196.09***	42.58***	2.38***	1N	10.4^{a}	9.0^{a}	9.4^{a}	7.5^{a}	9.7^{a}	5.7^{a}	5.5^{a}	6.3^{a}	8.2^{a}	8.8^{a}	6.0^{a}	4.7^{a}	7.7^{a}
				1/2N	11.8^{a}	11.4^{a}	9.1^{a}	7.0^{a}	10.3^{a}	5.9^{ab}	5.8^{a}	6.4^{a}	9.3^{a}	8.1^{a}	5.4^{a}	4.4^{a}	7.7^{a}
				1/4N	8.1^{b}	8.9^{a}	6.0^{b}	5.3^{b}	7.9^{b}	4.4^{c}	4.1^{b}	5.0^{a}	5.6^{b}	6.2^{b}	4.1^{b}	3.4^{b}	5.3^{b}
				1/8N	4.9^{c}	5.3^{b}	4.3^{c}	3.9^{c}	4.5^{c}	2.9^{d}	2.9^{c}	3.4^{b}	3.3^{c}	4.1^{c}	2.7^{c}	2.8^{c}	3.3^{c}
Internode Length [cm]	29.60***	29.11***	1.18$^{n.s.}$	1N	1.6^{a}	1.6^{ac}	1.5^{ab}	1.1^{a}	1.5^{a}	0.9^{a}	0.8^{a}	1.2^{a}	1.2^{ab}	1.5^{a}	1,1^{a}	1.0^{a}	1.4^{a}
				1/2N	1.9^{b}	2.1^{b}	1.6^{a}	1.2^{a}	1.7^{a}	1.2^{b}	1.0^{a}	1.3^{a}	1.4^{b}	1.7^{a}	1.1^{a}	1.2^{a}	1.6^{a}
				1/4N	1.7^{ab}	2.0^{ab}	1.3^{b}	1.2^{a}	1.6^{a}	1.1^{ab}	0.9^{a}	1.3^{a}	1.2^{ac}	1.6^{a}	1.0^{ab}	1.1^{a}	1.5^{a}
				1/8N	1.5^{a}	1.5^{c}	1.3^{b}	0.9^{a}	1.0^{b}	0.9^{a}	0.8^{a}	1.1^{a}	0.9^{c}	1.2^{b}	0.9^{b}	1.2^{a}	1.1^{b}
Chlorophyll Content [SPAD Values]	815.67***	68.68***	8.53***	1N	60.5^{a}	55.4^{a}	53.0^{a}	48.2^{a}	54.3^{a}	62.3^{a}	56.6^{a}	52.5^{a}	63.2^{a}	58.2^{a}	52.9^{a}	46.3^{a}	62.2^{a}
				1/2N	60.3^{a}	50.0^{b}	50.6^{a}	45.4^{a}	52.9^{ab}	57.2^{b}	51.1^{b}	48.9^{b}	58.5^{a}	48.6^{b}	43.2^{b}	35.2^{b}	50.5^{b}
				1/4N	47.9^{b}	43.1^{c}	37.7^{b}	29.4^{b}	49.6^{b}	43.4^{c}	38.7^{c}	30.3^{c}	37.8^{b}	32.7^{c}	27.6^{c}	26.3^{c}	26.4^{c}
				1/8N	46.0^{b}	38.7^{d}	25.2^{c}	21.7^{c}	40.5^{c}	37.0^{d}	35.0^{d}	21.5^{d}	30.6^{c}	24.6^{d}	22.8^{d}	21.2^{d}	14.9^{d}

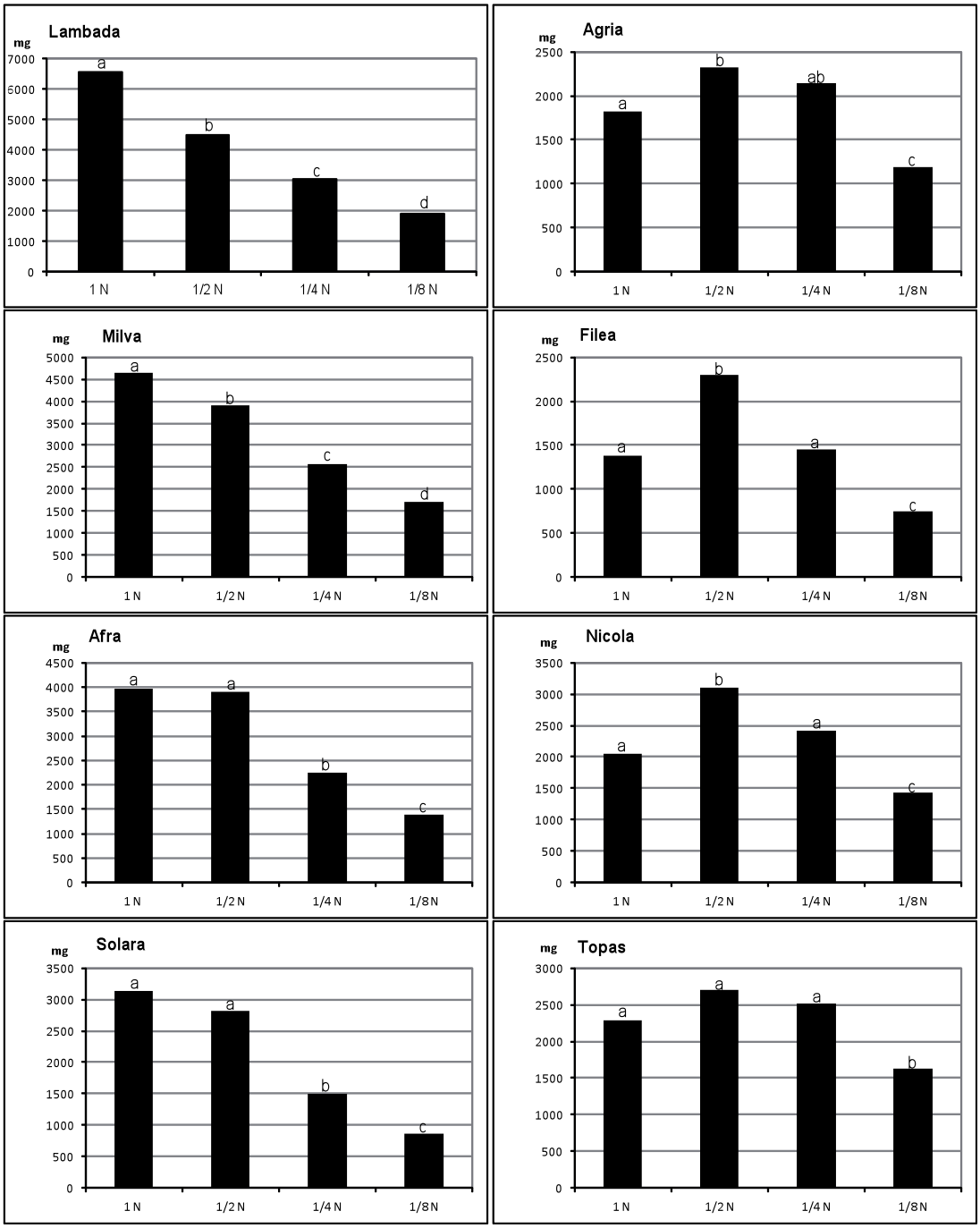


Fig. 1: Examples for differential genotype specific root response to nitrogen deficiency stress: Fresh matter of roots / ten plantlets after three weeks of culture in media with different levels of nitrogen. (Bars marked with different letters are significantly different according to LSD based multiple comparison test [p=0.05]; scales of y axes are different due to substantial differences in root development between cultivars.)

explanations are given in the captions to Fig. 2 and 3.

The distribution of data points in Fig. 2 illustrates that both nitrogen availability as well as genotype account for the large variation in biomass production determined as dry matter of shoots (Fig. 2a), roots (Fig. 2b) and whole plantlets (Fig. 2c). The decrease in nitrogen supply from the 1/2 to the 1/4 and 1/8 level is clearly reflected by a reduction in biomass yield after three weeks of culture, however in a varying extent according to the genotype. Plotting of data from dry matter obtained from plantlets (Fig. 2c) allows the identification of three clusters of genotypes. Cultivars 'Lambada', 'Milva', 'Solara' and 'Afra' represent the group with highest biomass development under full and 1/2 nitrogen regime, while cultivars 'Agria' and 'Topas' are separated from an intermediate group by a low performance at full nitrogen availability. However, the latter show a significant decline only at the 1/8 N-level while the corresponding threshold values for 'Lambada', 'Milva', 'Solara' and 'Afra' are already reached at the 1/4 N-level. Biomass allocation between different plant parts reveals an extraordinarily high contribution of roots in case of 'Lambada' (Fig. 2b), while the shoot fraction is comparatively low in 'Topas' and 'Agria' (Fig. 2a).

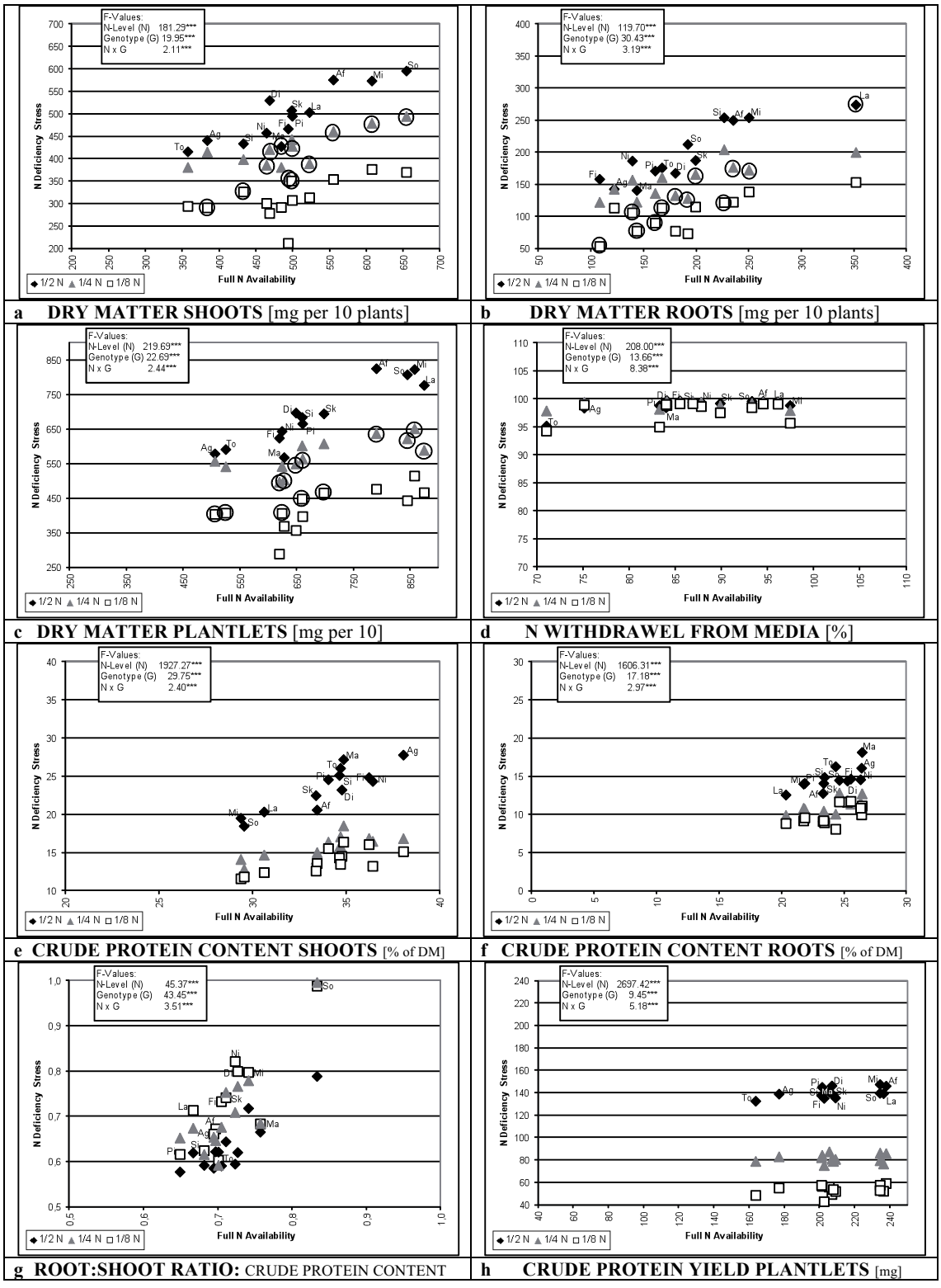
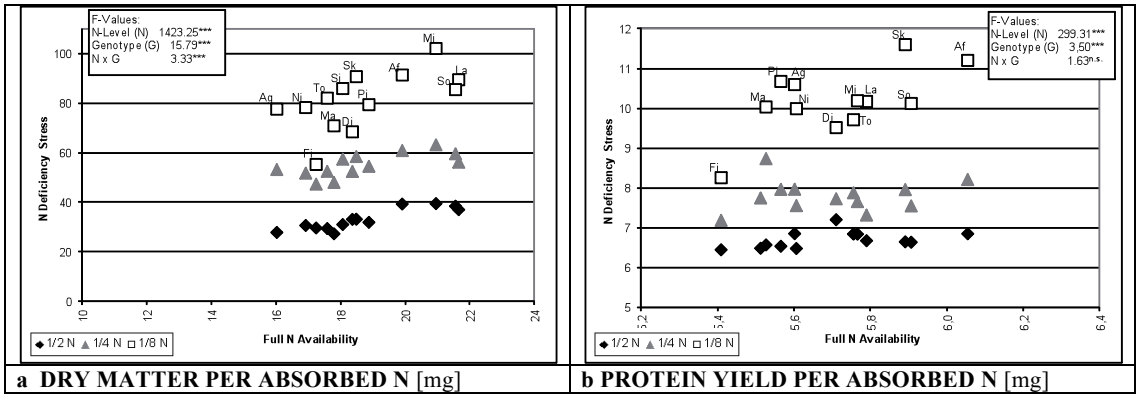


Fig. 2: Distribution of traits related to biomass, N-uptake, crude protein content and crude protein yield of 13 potato genotypes grown under three levels of nitrogen deficiency stress compared to full nitrogen supply. (Af = 'Afra'; Ag = 'Agria'; Di = 'Ditta'; Fi = 'Filea'; La = 'Lambada'; Ma = 'Marlen'; Mi = 'Milva'; Ni = 'Nicola'; Pi = 'Pirol'; Si = 'Simone'; Sk = 'Skala'; So = 'Solara'; To = 'Topas')
For each evaluated trait mean values determined under nitrogen deficiency stress (1/2 N, 1/4 N, 1/8 N) were plotted against values obtained at full nitrogen availability. This approach allows compiling of data from 13 genotypes and 4 nitrogen levels within one graph. Data points representing the 1N / 1/2N relationship are marked with the cultivar's name while corresponding data points for decreasing nitrogen availability are found in the perpendicular lines. The distribution and scattering degree of data points illustrate the impact of genotype and nitrogen availability on the modification of the evaluated characters. In addition, circles around data points indicate the nitrogen level causing a significant decline in the parameter in question for a given genotype. In this way, differences between cultivars in terms of response to nitrogen deficiency stress can be estimated by the position of specific threshold values.



a DRY MATTER PER ABSORBED N [mg] **b PROTEIN YIELD PER ABSORBED N** [mg]

Fig. 3: Distribution of traits related to nitrogen utilization of 13 potato genotypes grown under three levels of nitrogen deficiency stress compared to full nitrogen supply. (Af = 'Afra'; Ag = 'Agria'; Di = 'Ditta'; Fi = 'Filea'; La = 'Lambada'; Ma = 'Marlen'; Mi = 'Milva'; Ni = 'Nicola'; Pi = 'Pirol'; Si = 'Simone'; Sk = 'Skala'; So = 'Solara'; To = 'Topas')
For each evaluated trait mean values determined under nitrogen deficiency stress (1/2 N, 1/4 N, 1/8 N) were plotted against values obtained at full nitrogen availability. This approach allows compiling of data from 13 genotypes and 4 nitrogen levels within one graph. Data points representing the 1N / 1/2N relationship are marked with the cultivar's name while corresponding data points for decreasing nitrogen availability are found in the perpendicular lines. The distribution and scattering degree of data points illustrate the impact of genotype and nitrogen availability on the modification of the evaluated characters.

Nitrogen Uptake and Utilization

Fig. 2d - 2h present data of nitrogen withdrawal and nitrogen utilization associated traits. Generally all genotypes had used up the available nitrogen almost completely (93-97 %) after three weeks of culture in case of reduced N supply (1/2 N, 1/4 N, and 1/8 N). The situation is reflected by the short vertical distances between values determined for each genotype at the three levels of nitrogen reduction (Fig. 2d). However, significant differences between genotypes became evident in case of full nitrogen supply. Cultivars 'Agria' and 'Topas' are clearly separated from the rest of the genotypes due to a less efficient nitrogen uptake capacity. In contrast to the other genotypes, these cultivars have withdrawn only 75 and 71 % of the available nitrogen after three weeks of culture.

Highly significant differences were also determined between N-level, cultivars and genotype x nitrogen level interactions in case of crude protein related traits (Fig. 2e - 2h). Determination of crude protein contents in dry matter of shoots and roots explicitly reflects the nitrogen availability during the growth period (Fig. 2e and 2f). The decline between the two lowest N-levels (1/4 and 1/8 N), however, is less pronounced as obviously nitrogen supply in these media is immediately used up. Generally, crude protein concentrations in shoots are higher as compared to roots. However, the corresponding root:shoot ratios are increasing with decreasing nitrogen availability (Fig. 2g). In this context, cultivar 'Solara' holds a special position with comparable levels of crude protein in roots and shoots at the 1/4 N and 1/8 N-level, thus reaching an extraordinary high ratio. As a rule, crude protein concentrations were low in cultivars with high biomass production and vice versa.

With respect to crude protein yields of the whole plantlets three clusters of genotypes can be distinguished (Fig. 2h). These groups correspond exactly to the nitrogen uptake capacity of cultivars at full nitrogen availability. 'Topas' and 'Agria' can be distinguished by comparatively low, 'Solara', 'Milva', 'Lambada', and 'Afra' by high protein yields. The remaining seven cultivars constitute a distinct intermediate group.

Plots of dry matter produced per absorbed mg of nitrogen (Fig. 3a) as well as of protein yields per mg absorbed nitrogen (Fig. 3b) result in defined clusters according to the N-level, illustrating that the efficiency of converting nitrogen into biomass is steadily increasing with decreasing nitrogen availability. Cultivars 'Milva', 'Afra' and 'Lambada' are outstanding in terms of dry matter production at all N-levels, while biomass production of the normally vigorously growing cv. 'Solara' slightly declines upon reduction of nitrogen to 1/8 of the original concentration. 'Filea' performs generally poor, whereas 'Topas' and 'Agria' in spite of their low N-uptake capacity are not the poorest in dry matter production (Fig. 3a) and protein yield per absorbed unit of nitrogen (Fig. 3b) under nitrogen deficiency stress.

In order to compare the degree of nitrogen stress induced disturbances between cultivars, the stress susceptibility indices (SSI) were computed for dry matter and protein yield per available and per absorbed amount of nitrogen, respectively. Data is shown in Tab. 2 exemplarily for the 1/2 N-level. The SSI determines for each individual genotype the rate of change in a given trait between two environments relative to the mean change for all genotypes. The index is a measure for the extent of stability of a specific parameter under stress conditions, whereby high values indicate low tolerance. The high nitrogen uptake capacity of 'Lambada', 'Solara', 'Afra' and 'Milva' is reflected by low and the poor nitrogen acquisition of 'Topas' and 'Agria' by high SSI-values in case of productivity related to nitrogen availability. However, the latter two cultivars move to a higher ranking position when dry matter and crude protein yield are related to the amount of nitrogen actually absorbed from the culture medium. This indicates a comparatively more efficient metabolization under N limiting conditions.

Discussion

Nutrient use efficiency comprises two multifactorial components, i.e. utilization efficiency and uptake efficiency. While the first is attributed to the efficiency with which the nutrients are utilized to produce yield (agronomic efficiency) or biomass (physiological efficiency), the latter describes the effectiveness in absorbing nutrients from the surrounding environment (e.g. SATTELMACHER et al., 1994; LADHA et al., 1998; NAMAI et al., 2009). The experimental system used in this investigation allows the immediate measurement of nitrogen retrieval from the nutrient solution and the determination of the utilization efficiency in the sense of dry matter production and crude protein yield per mg of absorbed nitrogen within three weeks. Furthermore it enables the comprehensive monitoring of root

Tab. 2: Ranking of cultivars according to Stress Susceptibility Index **(SSI)** for dry matter (DM) and crude protein yield (PY) of plantlets per available and per absorbed nitrogen, respectively, produced on media with ½ N supply within three weeks.
The SSI (FISCHER and MAURER, 1978) determines for each individual genotype the rate of change in a given trait between two environments relative to the mean change for all genotypes, whereby high values indicate low tolerance.

DM per available N		DM per absorbed N		PY per available N		PY per absorbed N	
Lambada	0.52	Marlen	0,71	Lambada	0.77	Solara	0.64
Solara	0.56	Topas	0.89	Marlen	0.80	Lambada	0.77
Afra	0.66	Pirol	0.91	Solara	0.90	Nicola	0.80
Milva	0.73	Lambada	0.93	Milva	0.91	Afra	0.91
Nicola	0.86	Simone	0.95	Simone	0.97	Marlen	0.93
Skala	0.95	Filea	0.95	Skala	0.98	Filea	1.00
Marlen	1.06	Agria	0.97	Pirol	1.01	Ditta	1.05
Filea	1.06	Solara	1.03	Nicola	1.05	Simone	1.08
Simone	1.15	Skala	1.05	Afra	1.08	Agria	1.10
Ditta	1.20	Ditta	1.06	Filea	1.11	Skala	1.11
Pirol	1.28	Nicola	1.07	Ditta	1.14	Topas	1.15
Agria	1.67	Milva	1.16	Topas	1.24	Pirol	1.17
Topas	1.80	Afra	1.28	Agria	1.28	Milva	1.33

development under standard and nitrogen deficiency conditions.
Phenotyping of thirteen potato cultivars grown *in vitro* under four nitrogen levels revealed differential responses to N deficiency stress. All considered traits related to biomass production as well as to nitrogen uptake and utilization were significantly affected both by nitrogen availability and by the genotype. With a few exceptions, significant genotype x nitrogen level interactions were also detected.
Vegetative development, as measured by chlorophyll content, plant height, fresh and dry matter of shoots, roots and plantlets declined in all genotypes with reduction of the nitrogen supply. However, critical threshold levels associated with the impairment of specific traits varied with the cultivar. Notable differences between genotypes under all nitrogen regimes were particularly observed in root fresh matter production. While in some genotypes root fresh matter continually decreased with decreasing nitrogen availability, other cultivars intensified root development on the reduction of nitrogen supply to 1/2 of the original MS medium and were even able to sustain root proliferation at the 1/4 level. Changes in the root architecture, an increased root:shoot ratio and an increase of the root surface are well known responses of plants to nitrogen deficiency stress. DREW et al. (1973) demonstrated that locally restricted application of nitrate to primary roots of barley promoted a site-specific formation of lateral roots. In a further publication DREW and SAKER (1975) provided evidence for an associated increase in absorption and transport of 15N-labelled nitrate which appeared to compensate for the deficient supply at the remaining root system. The findings of these classical experiments have been supported manifold by corresponding investigations using diverse experimental systems and different plant species. In Arabidopsis such locally restricted nitrate dependent stimulation of lateral root growth has been attributed to the action of an ANR1 gene encoding specific transcription factors. It was predominantly expressed in roots and was induced within 30 minutes of nitrate application (ZHANG and FORDE 1998). In addition, there is evidence for a superordinated regulation system which restricts or allows proliferation of lateral roots dependent on the overall nitrate status of the whole plant (ZHANG et al., 1999). The situation has been discussed in detail by LEA and AZEVEDO (2006).
In our investigation plantlets were supplied with a liquid culture medium providing a homogeneous distribution and allowing a continuous uptake of nitrogen. Therefore, stimulation of root development under nitrogen deficiency stress observed in several genotypes has to be interpreted as a response to the nitrogen status of the whole plant. Such capacity of modifying root architecture in nitrogen limiting situations might be construed in terms of optimizing the nitrogen uptake efficiency. This view is supported by findings of SATTELMACHER et al. (1990) who recorded a significantly larger root system for the more N efficient genotype in a comparison of two potato cultivars differing in their nitrogen acquisition capacity. However, at this point it is not clear if such shift to preferential root proliferation upon limited nitrogen availability determined in our experimental system is a unique feature of specific cultivars. As evaluation of plantlets was generally performed three weeks after initiation of the experiments, the status of the plantlets at a particular moment in time was recorded irrespective of individual developmental rates. It is noteworthy that those cultivars which produced the highest amount of fresh matter are the ones which displayed a continuous reduction of root proliferation with decreasing nitrogen supply at the end of the three weeks' culture period (Tab. 1). Therefore, further experiments are undertaken in order to elucidate the state of root development at earlier time points.
Analysis of the residual nitrogen in the growth media after three weeks of culture resulted in the identification of three differentially responding groups of genotypes. 'Topas' and 'Agria' are characterized by a low nitrogen uptake capacity. In contrast, 'Afra', 'Lambada', 'Milva' and 'Solara' are distinguished from an intermediate group by their high nitrogen uptake capacity.
Under the specific experimental conditions nitrogen utilization efficiency is reflected by the dry matter production and by the protein yield per absorbed N. Generally, an explicit increase in nitrogen utilization efficiency with reduction of nitrogen availability was observed. These findings are in accordance with results of field and pot trials as well as with observations in natural habitats (e.g. GAUER et al., 1992; MA et al., 2004; YUAN et al., 2006). Dry matter production per mg absorbed nitrogen at the 1N level reflects the individual N-uptake capacity of genotypes (Fig. 3a, x-axis). With reduction of the nitrogen supply in the media the order of ranking is modified. While 'Afra', 'Lambada', and 'Milva' are still outstanding

in terms of nitrogen utilization efficiency, the performance of 'Solara' slightly drops. At the same time 'Filea' turns out to be the least efficient cultivar while 'Topas' and 'Agria' improve their relative performance under nitrogen deficiency stress (Fig. 3a, white squares). In conclusion, both genotypes characterized by a low N-uptake capacity are not the poorest with respect to N-utilization efficiency. The relative stability of the biomass production of the two cultivars under nitrogen deficiency stress is also reflected by evaluation of the SSIs at the 1/2 N supply level. This finding is in accordance with the different physiological processes and pathways underlying nitrogen uptake and subsequent metabolization (e.g. GOOD et al., 2004).

The crude protein content of shoots and roots decreased with the reduction of nitrogen in the culture media and, furthermore, was significantly determined by genotype and genotype x nitrogen level interactions. The crude protein concentration in both organs was negatively correlated with the amount of biomass produced, indicating a higher utilization capacity of strong growing genotypes with immediate metabolization of acquired nitrogen for proliferation of plantlets and production of plant material (SCHUM and JANSEN 2012). As a rule, the content of crude protein was lower in roots as compared to shoots. The comparatively steeper decline in shoots under nitrogen limitation resulted in increasing root:shoot protein ratios with decreasing nitrogen availability. The term crude protein comprises the total amount of organic nitrogen containing metabolites including true proteins, amines, amides, amino acids and peptides as well as nucleic acids. Determination of crude protein concentrations captures the nitrogen which was metabolized into organic compounds by the plantlets. However, it does not provide any information regarding the exact composition of the fraction. Nonetheless, the shift of the root:shoot ratios towards relatively higher values in roots may be a hint for reduced nitrogen transport rates into shoots under deficiency stress conditions. Especially cultivar 'Solara' stands out with almost equal levels of crude protein in roots and shoots at the 1/4 N and 1/8 N-level.

The identification of tolerances against abiotic stress factors under natural conditions in a crop like potato is difficult and requires a substantial input of time and labor. In addition, results of field trials are extremely affected by variable weather conditions between years. In this investigation a highly controlled environment was chosen for evaluation of potentially genotype specific responses to different degrees of nitrogen deficiency stress. *In vitro* cultures allow standardizing growth conditions and facilitate the manipulation of single factors. The potential of *in vitro* cultures for evaluation of potato germplasm with respect to salt, heat and drought tolerance has been discussed earlier (ANITHAKUMARI et al., 2011; ARVIN and DONELLY, 2008; DONELLY et al., 2003; KHRAIS et al., 1998 and references cited therein). On the other hand, important agronomic traits as tuber yields cannot be assessed. Furthermore, physiological conditions that differ from the natural situation have to be taken into account. In particular, plantlets are incubated under mixotrophic conditions and transpiration rates are reduced due to high humidity within the culture vessels. Therefore, such an approach is intended for initial screening of germplasm and identification of divergently responding genotypes for subsequent detailed investigations in pot and field experiments. Currently, attempts are made to correlate results of *in vitro* experiments to data obtained from potatoes grown under more natural conditions in a rain out shelter.

Application of the *in vitro* test system resulted in identification of differentially responding genotypes in terms of nitrogen acquisition and metabolization capacity under the given experimental conditions. Furthermore, particular cultivars with increased root development under nitrogen deficiency stress were determined. The characterization of individual components contributing to nitrogen use efficiency in potato may help to identify promising genotypes in breeding of N efficient potato cultivars. In addition, the experimental system is suited for investigation of early physiological responses to nitrogen deficiency stress under highly controlled conditions at the proteomic level.

Conclusion

An *in vitro* test system was developed which facilitates analyzing specific responses to nitrogen deficiency stress in potato within three weeks. The experimental system allows the immediate measurement of nitrogen retrieval from the nutrient solution and the determination of the utilization efficiency in the sense of biomass production and crude protein yield per mg of absorbed nitrogen. Genotype dependent differences were detected with respect to various morphological and physiological traits under nitrogen limiting conditions. Furthermore, the method enabled the prompt identification of genotypes responding with increased root development under nitrogen deficiency stress. This approach is helpful for initial screening of germplasm and identification of divergently responding genotypes as well as for investigation of early physiological responses to nitrogen deficiency.

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