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Classification of oilseed rape accessions according to sulfur-related plant traits in short-term experiments reflects agronomic performance in field experiments

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ABSTRACT

Sulfur (S) deficiency can negatively affect seed yield and nitrogen (N) use efficiency of winter oilseed rape. In this study, it was tested whether genotypic variation in sulfur-related plant traits exists and how this is related to agronomic performance of oilseed rape accessions at low and high N availability in the field. A range of highly diverse oilseed rape accessions was grown in short-term hydroponic experiments under N-limiting conditions. Accessions showed high variation in S uptake and S distribution within shoots, and particularly in leaf S concentration, malate:sulfate ratio and glucosinolate concentration, while variation in N-related plant traits was less pronounced. Accessions could be classified into four groups by cluster analysis using the measured plant traits in hydroponics. The same grouping of the accessions could also be achieved by using only the leaf S parameters. Apart from the variation in hydroponic experiments, groups also differed in agronomic parameters in the field. Within glucosinolate-containing accessions those characterized by high S uptake and S distribution in hydroponics showed superior yield both at low and high N supply. Low-glucosinolate (00) accessions with high S uptake in hydroponics had higher seed S concentrations than other 00-accessions in field experiments. It was concluded that assessing S-related plant traits under controlled conditions in an early growth stage is effective for identifying genotypes with superior S utilization.

1. Introduction

Oilseed rape is one of the most sulfur (S) demanding crops, with a need of around 17 kg S t^{-1} seed yield (De Kok et al., 2011) compared to 2 − 3 kg S t^{-1} in wheat (Zhao et al., 1999). Reasons for this high demand are ascribed to high protein and glucosinolate (GSL) contents in the seeds (Fismes et al., 2000), the S requirement for oil synthesis (Janzen and Bettany, 1984) and to a high sulfate concentration in the biomass (McGrath and Zhao, 1996), which is poorly remobilized (Hawkesford, 2000). Due to this high demand and to decreasing S supply to crops by atmospheric deposition as well as increased S removal of high-yielding crops, S deficiency has apparently become a widespread problem in oilseed rape production (Grant et al., 2012; Sarda et al., 2014).

A particular problem of S deficiency is its negative impact on the N use efficiency of crops (Fismes et al., 2000). S deficiency can down-regulate nitrate uptake and N translocation to the shoot (Clarkson et al., 1989). Decreased N uptake at S deficiency has also been observed on the field level (Fismes et al., 2000; Salvagiotti et al., 2009). In addition, nitrate reductase as well as glutamine synthetase transcripts and activities are reduced under S starvation, leading to nitrate and amino acid accumulation (Prosser et al., 2001; Kaur et al., 2011). This contributes to the down-regulation of nitrate uptake (Kaur et al., 2011). Furthermore, high levels of toxic N metabolites may result in seed yield depressions that occur at high N/S supply ratios (Janzen and Bettany, 1984).

On the other hand, it is also possible that N deficiency negatively affects S uptake and utilization. Several points in S metabolism might be disturbed under N-limiting conditions: Sulfate uptake can be down-regulated under N limitation due to low levels of O-acetylserine (OAS) (Buchner et al., 2004). OAS as a precursor of cysteine (Hesse et al., 2004) is decisive for the S assimilation pathway and less abundant under N limitation (Kim et al., 1999). N deficiency also downregulates several steps of the S assimilation pathway, presumably at the transcript level via OAS (Kopriva and Rennenberg, 2004; Davidian and Kopriva, 2010), so that sulfate might accumulate under these conditions.

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Although sulfate is an important source for S remobilization during high S demand (Sunarpi and Anderson, 1997; Hawkesford, 2000), sulfate remobilization in oilseed rape was found to be very slow and might therefore be insufficient to fulfil the demand (Blake-Kalff et al., 1998; Hawkesford, 2000). On the other hand, early leaf senescence and proteolysis will occur under N deficiency, and S remobilization can be increased along with N remobilization (Sunarpi and Anderson, 1997; Dubousset et al., 2009).

In a study comparing S utilization in oilseed rape cultivars at low and high N supply, no general reduction in shoot S concentration and S assimilation has been found at low N supply (Wang et al., 2016). According to the above-mentioned studies, S remobilization from senescing leaves was enhanced. However, there was considerable genotypic variation in S uptake and S remobilization at low N supply that was not found at high N supply. Therefore, depending on the cultivar, S deficiency problems might occur at limiting N supply, despite a sufficient S fertilization level.

Under these circumstances, eliminating S deficiency just by fertilization is not the most economic and environmentally favorable way. Given the strong genotype by environment interactions, it will not be an easy task to properly adjust fertilizer rate, which might lead to over-fertilization in one case and to nutrient limitation in another. The identification and breeding of genotypes that are able to efficiently absorb and utilize S therefore presents a more sustainable and secure long-term approach (Balint and Rengel, 2011).

Genotypic variation in S uptake and utilization in oilseed rape have not been studied intensively so far, apart from an Australian study starting with eighty-four genotypes. Genotypic variation in S efficiency parameters was found in this study (Balint et al., 2008), that could be traced back to variations in S uptake and S remobilization (Balint and Rengel, 2011).

The objective of this study was (i) to identify genotypic variation of oilseed rape in S uptake and utilization in short-term experiments and (ii) to evaluate the consequences of this variation for yield and N efficiency in the field. It was hypothesized that genotype selection in short-term experiments is feasible and that superior S uptake and utilization of individual accessions can contribute to high yields especially under N-limiting conditions.

2. Material and methods

2.1. Hydroponic experiments

32 oilseed rape accessions were raised in short-term experiments in a greenhouse in hydroponics with limiting N and sufficient S supply. The accessions present a broad genetic background including different germplasm types, seed quality types, origins, and release periods (Table 1). They had previously been selected from a diversity set based on genetic data (Bus et al., 2011; Stahl et al., 2016). Seeds were supplied by Norddeutsche Pflanzenzucht Lembke (NPZ, Hohenlieth, Germany). Four individual experiments, each presenting one replicate and lasting for 62–66 days, were performed between October 2012 and June 2013 at a day/night temperature setting of 25 °C/18 °C and a relative humidity of 75%.

Four seedlings from each accession were transplanted into continuously aerated nutrient solution in 10 l pots and thinned to two seedlings per pot after 8 days. Nutrient solution was supplied at first at 25% strength, increased to 50% strength on day 3 and to full strength on day 5. Full strength nutrient solution contained 1000 µM KCl, 500 µM $CaCl_2$, 200 µM K_2HPO_4, 75 µM KH_2PO_4, 300 µM $MgSO_4$, 10 µM H_3BO_3, 2 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$, 0.05 µM $(NH_4)_2Mo_7O_{24}$ and 60 µM Fe-EDTA. N supply was 0.5 mM N (80% $Ca(NO_3)_2$ and 20% NH_4NO_3). It was decreased to 0.1 mM N when the third leaf (leaf 3) counted from the base of the plants appeared, and further decreased to 0.05 mM N when leaf 3 was fully expanded (18–22 days after transplanting). Additional $CaCl_2$ solution was used to complement Ca supply to the set concentration (1 mM). Nutrient solution was replaced three times a week to maintain the set concentrations.

Senescence of leaf 3 was recorded by SPAD measurements (SPAD-502, Minolta, Japan) performed on every second day from leaf expansion until complete leaf yellowness (SPAD = 0). Timing of full leaf expansion was identified by leaf width measurements on every second day.

Harvests were done individually for each accession when leaf 3 was shed from both plants in the pot. Depending on accession and experiment, this was the case between 42 and 64 days after transplanting. After harvest, shoots were separated into leaf 3, the youngest fully expanded leaf, developing leaves and the rest shoot. Fractions were freeze-dried to constant weight and ground to fine powder for further measurements.

Total N and S concentrations of each fraction were determined according to the Dumas combustion method using a CNS elemental analyzer (Flash EA 1112 NCS, Thermo Fisher Scientific, Waltham, MA, USA). Shoot N and S uptake was calculated as N or S concentration of each plant fraction multiplied by dry weight of the respective fraction, and then summed up for all fractions (leaf 3, youngest fully expanded leaf, developing leaves and rest shoot).

Sulfate and malate concentration was measured by ion-chromatography (ICS 2500, Dionex, Sunnyvale, CA, USA). Anions were extracted with de-ionized water in a boiling water bath for 5 min. After incubation on ice for at least half an hour, samples were centrifuged and proteins were further excluded from the supernatant by chloroform extraction. Supernatant was filtered on C18-columns (Strata spe. 8B-S001-DAK) prior to ion-chromatography.

GSLs in youngest fully expanded leaves were measured according to the method of Krumbein et al. (2005) with slight modifications. 0.2 g freeze-dried powder were extracted with 2 ml of 70% boiling methanol (10 min, 75 °C water bath) with 100 µl of 5 mM sinigrin solution (Sigma-Aldrich Co., MO, USA) as internal standard. Afterwards, 0.5 ml barium acetate (0.4 M) was added to precipitate proteins. After centrifugation (4000 rpm, 10 min, 25 °C), the pellets were re-extracted twice. Supernatants were combined and made up to 5 ml with 70% methanol. 4.5 ml extract was loaded onto a 1 ml mini-column (JT Baker, USA) containing 200 µl of activated DEAE Sephadex™A25 (Amersham Biosciences, Sweden). Bound GSLs were de-sulphated with aryl sulfatase (Sigma-Aldrich Co., MO, USA) over night. The desulfo-GSL were eluted with 5 ml bi-distilled water. Samples were analyzed by HPLC (LC-10AT pump, CTO-10A column oven, SCL-10A VP system controller, Shimadzu, Kyoto, Japan) consisting of a UV–VIS detector (SPD-10A) set at 229 nm and a prontosil ODS2 column (250 × 4 µm, 5 µm, Bischoff, Germany). The mobile phase consisted of ultra-pure water and 20%/100% acetonitrile (Tedia, USA) with a flow rate of 1.3 ml min^{-1}.

Total glutathione (glutathione (GSH) plus glutathione disulfide (GSSG)) in youngest fully expanded leaves was measured according to Griffith (1980) using an enzymatic method with GSSG solution as a standard.

Plant traits used for the classification of accessions were the following:

Shoot dry weight (SHDW), shoot N and S uptake (SHN, SHS), shoot N and S concentration (SHNC, SHSC), shoot N:S ratio (SHOOTNS), shoot sulfate-S:S ratio (SHSULSR), as well as corresponding values for the youngest fully expanded leaf (YL) at harvest (YLNC, YLSC, YLNS, YLSULSR). For YL, malate:sulfate ratio (MALSUL) as an indicator of S deficiency (Blake-Kalff et al., 2000) was calculated on a molar basis, and total GSL and glutathione (GLUT) were analyzed.

S distribution (SDISTR) was calculated as S concentration in developing leaves divided by YLSC. S remobilization (SREM) was calculated as S concentration in the dead leaf 3 divided by SHSC. NDISTR and NREM were calculated accordingly.

Senescence duration (SENDUR) was defined as the time between