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ORIGINAL ARTICLE

Selection of plant physiological parameters to detect stress effects in pot experiments using principal component analysis

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Abstract
Appropriate selection and well-timed measurement of plant developmental, morphological and physiological parameters are essential to maximize efficacy and minimize time consumption of experiments. To select for the most sensitive indicators of drought or salt stress, three independent pot experiments with diverse setups were analysed with 20–20 measured parameters. Parameters of plant growth, phenology and symbiotic interactions, visual stress symptoms, photosynthetic activity, nutrient composition and vitality were studied and the result matrices were evaluated with principal component analysis (PCA). Stress effects manifested in PC1 of two experiments and in PC2 of the third one. Traits assumed to be adequate for stress indication were characterized by high PC1 or PC2 loading values. Beside parameters of biomass production, growth and visible stress symptoms, less evident traits e.g. root electrical capacitance, membrane stability index in roots and leaves, relative water content of leaves and SPAD units were identified.

Keywords Plant trials · Stress indication · Drought · Salinity

Introduction

Stress is an altered physiological response of living organisms caused by physical, chemical or biotic environmental factors that tend to shift their equilibrium away from its optimal thermodynamic state (Gaspar et al. 2002; Strasser 1988). Soil or water pollution, climate change or other anthropogenic effects can cause severe abiotic or biotic stress for both cultivated plants and natural vegetation. Understanding the effective plant resilience and adaptation to heterogeneous and changing environmental conditions is, therefore, in the forefront of agricultural, ecological or conservation research.

An appropriate experimental design with a selection of the most sensitive parameters to be measured is a prerequisite for an effective and time efficient study, although the right choice of these parameters is not always obvious. Regarding their relevance, applicability and the adequate number of parameters to be used, there are numerous examples of experimental approaches. Several groups of quantitative or qualitative parameters exist which have been applied to characterize plant development and growth, physiological status, symbiotic interactions, stress symptoms, photosysnthesis, etc. during or at the end of an experimental growth period (Berger and Ludwig 2014; Grümberg et al. 2015; He and Dijkstra 2014; Kalaji et al. 2016; Latef and Chaoxing 2014; Munns 2002; Roger 2001; Salvatori et al. 2014; Talaat et al. 2015; Wehner et al. 2015; Zhang et al. 2015): (1) the simplest and most obvious parameters are: fresh and dry weight, root and shoot biomass production, root to shoot ratio, leaf area, grain yield, reproductive index. Most of them can be measured in a non-destructive manner, such as using optical imaging techniques, e.g., plant height, shoot diameter, leaf number, number of nodes, colour of leaves, the state of flowering, podding or grain filling, as well as observations of growth morphological dynamics, visible impacts of stress-based

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wilting symptoms, senescence, leaf necrosis, and phenotypical variations (Berger et al. 2010; Li et al. 2014; Vollenweider and Günthardt-Georg 2005). (2) Plant responses characterized by the nutritional status of plant shoots, roots or yield. (3) Water relations of the plant: leaf water potential, relative water content in leaves, absolute and relative transpiration rates (Verslues et al. 2006). (4) Parameters related to photosynthesis (Chaves et al. 2009) have been previously measured by destructive methods, such as chlorophyll concentration or intracellular CO_2 concentration in leaves, whereas they are already determined mostly non-destructively and can be measured several times during an experiment, e.g., chlorophyll fluorescence measurements (Krause and Weis 1984), SPAD (Soil Plant Analysis Development) units, stomatal conductance or photosynthetic water use efficiency. (5) Protein, free amino acid, proline, glycin-betaine, soluble sugar and endogenous abscisic acid content of plant tissues or metabolic fingerprinting values (Schauer and Fernie 2006; Shulaev et al. 2008) describe plant biochemical processes, and they are potential stress indicators. (6) Measurement of enzyme activities, e.g., ATPase, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, or characterization of oxidative damage or lipid peroxidation are also sensitive tools on the biochemical level. (7) Electrolyte leakage and membrane stability indices may provide valuable information about the condition and the potential resilience of plant cells. (8) The presence or absence and the developmental state of symbiotic relationships may indicate plant physiological status and sign stress effects indirectly (Füzy et al. 2008a). (9) Measurement of electrical capacitance in root–soil systems is a promising non-destructive method for assessing root growth and activity (Cseresnyés et al. 2013). (10) The study of the dynamics of plant metabolism and regulatory mechanisms under stress often requires a combination of the traditional physiological approaches with functional genomic characterization using transcriptomic, proteomic, metabolomic or ionomic analysis (Chaves et al. 2009).

A joint multivariate statistical analysis on many of these plant growth or physiological parameters used in three independent experiments with different test plants and environmental conditions is presented. Our analysis aimed to assess the adaptability of these parameters to indicate stress responses.

Materials and methods

Pot experiments

Three independent pot experiments (I–III) were accomplished. In experiment (I) salt tolerance of two wheat (*Triticum aestivum* L.) cultivars were tested, in experiment (II) two soybean (*Glycine max* L.) varieties were grown under drought stress conditions, while in experiment (III) sea aster (*Tripolium pannonicum* L.), a common halophyte was grown under drought, salt and combined stress conditions (Tables 1, 2).

Measured parameters

The following parameters were measured during the growth period of plants or at the end of the experiments—some of them were measured only in 1 or 2 experiments (Table 2).

Plant growth parameters: the oven dried (70 °C, 72 h) plant root and shoot biomass (SDM, RDM), root:shoot ratio (R/S) and leaf area (LA) were measured and calculated at harvest, while, plant height (PH), leaf number (LN), number of nodes (NN), length of the longest leaf (LL) and the presence of flowers (FLW) were monitored during the total plant growth period.

Visual assessment of plant condition: colour of leaves (COL), wilting symptoms (WS) after drought stress were surveyed and categorized in a four grade scale: 0: no visible symptoms, 1: 1–2 affected leaves, 2: serious wilting on some leaves, 3: wilting symptoms on whole plant, some leaves die away, 4: plant death. Number of dry leaves (DL) was counted regularly, while relative water content of leaves (RWC) was measured more times during the experiments (Barrs and Weatherley 1962; Gonzalaz and; González-Vilar 2007).

Functional parameters of the photosynthetic apparatus: chlorophyll content (CHL) was determined according to Porra et al. (1989). Stomatal conductance (SC) was measured by a leaf porometer (Decagon Devices Inc., Pullman, WA, USA; Model Sc-1). Photochemical activity of photosystem II characterised by F_v/F_m and F_v/F_0 values were measured by a Chlorophyll *a* fluorometer (Opti-Sciences OS-30p + Fluorometer, Hudson, New Hampshire, USA, Tsimilli-Michael and Strasser 2008). SPAD values were monitored by a SPAD-502 meter (Konica Minolta Inc., Osaka, Japan).

Electrical capacitance (EC) and phase angle of the impedance (*F*) were measured in the root–soil system. EC is determined by the extension and the uptake activity of the root system (Cseresnyés et al. 2016), while F primarily depends on the physicochemical properties of root tissues (Cseresnyés et al. 2013). Capacitance response of the root–substrate system was detected using a GW-8101G precision LCR meter (GW Instek Co. Ltd., Taiwan) at 1 kHz and 1 V AC. EC and F values for parallel resistor–capacitor (RC) circuit were displayed. The ground electrode (a stainless steel rod, 15 cm long and 6 mm i.d.) was inserted into 10 cm depth in the substrate at 5 cm distance from the stem base, while the plant electrode was attached to the stem at

Table 1 The term, conditions and measured parameters of the three pot experiments

	Experiment I	Experiment II	Experiment III
Plant	Wheat (*Triticum aestivum* L.)	Soybean (*Glycine max* L.)	Sea aster (*Tripolium pannonicum* L.)
Cultivars	1 tolerant 1 sensitive	2 Hungarian variety Emese, Alíz	–
Other treatments	–	–	Inoculation with AM fungi: G, A, L
Parallels	10	10	3
Substrate	Rhyolite-vermiculite mixture	Calcic chernozem soil	Pumice
Volume per pot	700 mL	700 mL	150 mL
Duration	40 days	65 days	140 days
Stress factors	Salt—Na_2CO_3 Three doses: 0.1, 0.2, 0.3 m/m % in substrate—added before seeding	Drought—reduced watering until wilting point, 2×2 week period	Salt—Na_2CO_3 (0.1%) Drought—PEG (2.5%) Salt and drought (watering weekly)
Nutrient addition	Hoagland solution weekly	–	Hoagland solution weekly
Conditions	16/8 h photoperiod (400–500 µmol/m^2/s), 26/18 °C, respectively		
Measured parameters	EC1, EC2, EC3, EC4, SDM, RDM, F1, F2, F3, F4, PH6, PH12, R/S, LA, MSIL, TTC, F_v/F_m, F_v/F_0, SPAD, SC	EC, SDM, RDM, PH, LA, R/S, NN, WS, RWC, F_v/F_m, CHL, M, A, NOD, N, P, K, Fe, Mn, Zn	SDM, RDM, LL, R/S, LN2, LN4, COL, DL, RWC, FLW, ET, MSIL, MSIR, TTC, M, A, F_v/F_m, SPAD3, SPAD6, SPAD9

G Funneliformis geosporum strain (BEG47), *A* soil with indigenous AM fungi from artemisia steppe, *L* soil with indigenous AM fungi from short grass pasture, the soils were collected from a salt effected soil (Hungary, Apaj), *EC* root electrical capacitance, *F* phase angle, *SDM* shoot dry mass, *RDM* root dry mass, *PH* plant height, *LA* leaf area, *R/S* root/shoot ratio, *LL* leaf length, *DL* number of dry leaves, *NN* nodes number, *LN* number of leaves, *COL* leaf colour, *WS* wilting symptoms, *FLW* flowering stage, *RWC* relative water content of leaves, *MSIL* membrane stability index of leaves, *MSIR* membrane stability index of roots, *TTC* root vitality test, *CHL* chlorophyll content, *SPAD* SPAD units, F_v/F_m calculated chlorophyll fluorescence data, *SC* stoma conductance, *ET* ethylene production of roots, *M* intensity of AMF colonization, *A* arbuscule richness in roots, *NOD* root nodulation rate, *N, P, K, Fe, Mn, Zn* macro- and micro-element content of shoots, *1–9 after the letters* weeks after stress treatment started

equal height (1 cm) above substrate level through a 5-mm-wide aluminium strip. 2 h before electrical measurements, pots were watered to field capacity.

Macro- and micronutrient concentrations in plant tissues were also investigated. P, K, Zn and Fe concentrations were assessed after wet digestion of the air-dried plant samples with cc. HNO_3 + cc. H_2O_2. Shoot nutrient contents were measured by an ICP-AES instrument (Jobin–Yvon, ULTIMA2). Nitrogen content of the leaves was determined by the Kjeldahl method (1883) after digestion of the samples in sulfuric acid (cc. H_2SO_4).

Root nodulation rate (NOD) of soybean plants were scored from 0 to 8. 0: no nodules, 1: 1–10 nodules, 2: several nodules, but low density, 3: nodules are in medium density, 4: very dense nodulation. The assessments were made for the main root and the lateral roots separately, then the scores were summarized. Arbuscular mycorrhizal fungal (AMF) colonization of roots were determined after clearing and staining (Phillips and Hayman 1970). Fungal colonization intensity (M) and arbuscule richness of roots (A) were calculated according to Trouvelot et al. (1989).

Vitality and biochemical parameters were measured as well: the membrane stability index in roots (MSIR) and/or shoots (MSIS) (Sairam et al. 1997), root vitality test by the triphenyl-tetrazolium chloride (TTC) method (Clemensson-Lindell and Persson 1995) and the root ethylene production (ET) by GC (Bassi and Spencer 1989; Cristescu et al. 2012).

Statistical analyses

Principal component analyses (PCA) were made by 20 selected variables from each experiment to designate the main factors that indicate stress situations effectively. The statistical analyses were carried out by the Statistica software package (Dell Inc. 2015. version 13).

Results

As a result of the PCA, the projections of the cases on a factor-plane with the two principal components (PC) were imaged (Figs. 1, 2, 3a), and the efficiency of separation in case of the main factors were checked. A very clear separation of salt treatments was shown by PC1 in experiment I, which accounted for 45.7% of the total variation. Control and salt treatments were separated with minimal overlapping (Fig. 1a). Statistical analysis of experiment

Table 2 Abbreviation, time of measurement and measurement characteristics of the plant parameters examined

Parameters	Abbr.	Experiment			Unit	Instrument	Reference	Measurement specification
		I	II	III				
Shoot dry mass	SDM	H	H	H	g plant^{-1}			
Root dry mass	RDM	H	H	H	g plant^{-1}			
Plant height	PH	G	H	–	cm			
Leaf area	LA	H	H	–	cm^2	Canon, LIDE120		
Root: shoot ratio	R/S	H	H	H				
Leaf number	LN	–	–	G	pcs			
Leaf length	LL	–	–	G	cm			Length of the longest leaf
Node number	NN	–	G	–	pcs			Main- and lateral-stem nodes
Wilting symptoms	WS	–	G	–				Visual assessment (0–4 scale)
Dry leaf number	DL	–	–	G	pcs			
Leaf colour	COL	–	–	G				Visual assessment (1–3 scale)
Relative water content	RWC	–	G	H	%		Barrs and Weatherley (1962)	G: measured at the end of the first drought period
Flowering stage	FLW	–	–	G				Presence or absence of flowers or flowering stem
Maximal quantum efficiency	F_v/F_m	H	–	H		Opti-Sciences, OS-30p fluorometer	Tsimilli-Michael and Strasser (2008)	
Chlorophyll fluorescence	F_v/F_0	H	–	H		Opti-Sciences, OS-30p fluorometer	Tsimilli-Michael and Strasser (2008)	
Chlorophyll content	CHL	–	H	–	µg g^{-1}	Helios β, spectrofotometer	Arnon (1949) Porra (1989)	Chlorophyll a + b
SPAD value	SPAD	H	–	G		Konica Minolta, SPAD-502		
Stomatal conductance	SC	G	–	–	mmol m^{-2} s^{-1}	Decagon Devices, Sc-1		
Membrane stability index of roots	MSIR	H	–	H	%		Sariam et al. (1997)	
Membrane stability index of leaves	MSIL	–	–	H	%		Sariam et al. (1997)	
Root vitality	TTC	H	–	H	abs g^{-1}	Helios β, spectrofotometer	Clemensson-Lindell and Persson (1995)	
Ethylene production	ET	–	–	H	nmol g^{-1} h^{-1}	GC 8000, Fisions instruments	Bassi and Spencer (1989)	
Nitrogen	N	–	H	–	%		Kjeldahl (1883)	Digestion in cc.H_2SO_4
Phosphorus	P	–	H	–	mg kg^{-1}	ICP-AES, ULTIMA2		Digestion in cc.HNO_3 + cc. H_2O_2
Potassium	K	–	H	–	mg kg^{-1}	ICP-AES, ULTIMA2		Digestion in cc.HNO_3 + cc. H_2O_2
Iron	Fe	–	H	–	mg kg^{-1}	ICP-AES, ULTIMA2		Digestion in cc.HNO_3 + cc. H_2O_2
Manganese	Mn	–	H	–	mg kg^{-1}	ICP-AES, ULTIMA2		Digestion in cc.HNO_3 + cc. H_2O_2
Zinc	Zn	–	H	–	mg kg^{-1}	ICP-AES, ULTIMA2		Digestion in cc.HNO_3 + cc. H_2O_2
Electrical capacitance	EC	G	H	–	nF	GW-8101G, LCR meter	Cseresnyés et al. (2016)	1 V, 1 kHz (AC)
Phase angel	F	G	–	–	Degree	GW-8101G, LCR meter	Cseresnyés et al. (2013)	1 V, 1 kHz (AC)
AMF—M%	M	–	H	H	%	Olympus BX51 microscope	Trouvelot et al. (1989)	Root colonization intenzity

Table 2 (continued)

Parameters	Abbr.	Experiment			Unit	Instrument	Reference	Measurement specification
		I	II	III				
AMF—A%	A	–	H	H	%	Olympus BX51 microscope	Trouvelot et al. (1989)	Arbuscule richness
Bradirhizobial nodulation	NOD	–	H	–				Visual assessment (0–4 scale on main and lateral roots)

G the parameter was measured one or more times during the growth period, *H* the parameter was measured only at harvest

II with drought stress resulted in a very similar pattern: control and drought stressed plants were separated according to PC1 without any overlap, where PC1 accounted for 39.4% of the total variation (Fig. 2a). Though with a strong overlap, there is a clear bias between the two wheat varieties along PC2 in experiment I, while no separation between soybean varieties were detected in experiment II. In experiment III with sea aster plants, drought, salt and combined stress effects seemed to show a limited separation along PC2—control cases tended to appear towards the higher values (Fig. 3a). PC1 of experiment III separated the plants according to the origin of the microbiota of the inoculum used, i.e., one of the two halophyte communities or a pure culture (Table 1). Microbial inoculation, therefore, caused higher variation in the measured parameters, than stress treatments, which accounted only for 16.5% of the total variation along PC2.

The traits adequate for stress indication can be chosen according to their higher loading values on PC1 of experiment (I) and (II) and PC2 of experiment (III) (Figs. 1, 2, 3b). Among the 20 measured traits in PC1 of experiment I, electrical capacitance values of the root-soil system, shoot and root dry weights and plant height measured at 6 weeks old plants were responsible for the maximum variation. The maximum variation of PC1 in experiment II originated from values of shoot dry weight, relative water content of leaves, electrical capacitance, wilting symptoms, leaf area and plant height at harvest. At experiment III the distribution of variation among traits was analysed in PC2 (Fig. 3b). The maximum variation along PC2 of experiment III was explained by leaf numbers of plants measured 2 and 4 weeks after stress treatment started, SPAD value, membrane stability index in leaves and roots and number of dry leaves 7 weeks after stress treatment started.

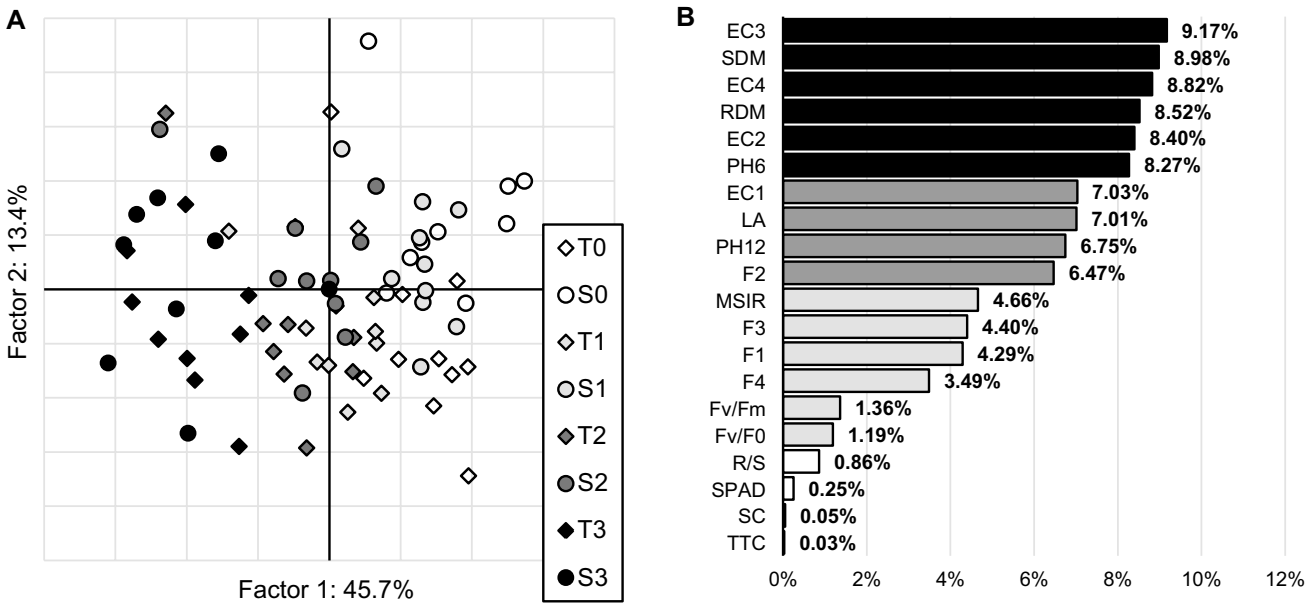

Fig. 1 The result of PCA in experiment I. **a** Projection of the cases on factor-plane (T/S: wheat varieties—tolerant/sensitive, 0–3: salt doses), **b** the PC1 loading values of the 20 measured parameters (abbreviations of the traits are in Table 1)

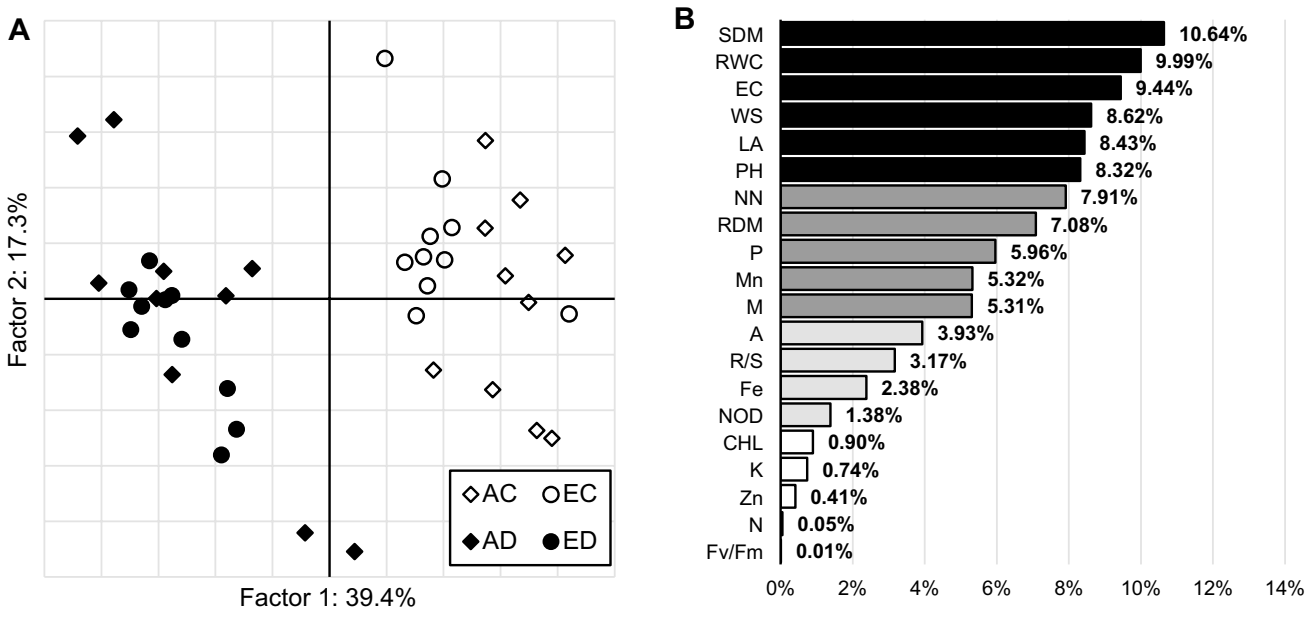

Fig. 2 The result of PCA in experiment II. **a** Projection of the cases on factor-plane (A/E: soybean varieties—Alíz/Emese, C/D: control/drought stressed plants), **b** the PC1 loading values of the 20 measured parameters (abbreviations of the traits are in Table 1)

Discussion

Drought and salinity stresses generally reduce plant growth and productivity by decreasing e.g., CO_2 assimilation rates and stomatal conductance, reducing leaf area, stem extension and root growth, or disturbing plant osmotic relations and water-use efficiency (Farooq et al. 2009; James et al. 2008; Munns 2002; Ohashi et al. 2006; Ueda et al. 2003). To manage multiparametric data matrices and select treatments responsible for the maximal variety, PCA is a useful tool, which can be a guidance among trials (Dresler et al. 2014; Maruyama et al. 2014; Chen et al. 2014; Sutka et al. 2011).

In our study, stress treatments were clearly separated both in experiment I and II, as salinity and drought caused a much higher variance in parameters, than the plant cultivar or any other circumstances. AMF inoculation contributed to the highest variance in experiment III (sea aster, drought and salt stress), while control and stressed plants were separated along the y axis in a limited degree (Fig. 3a). A slight shift in the microbial community can cause substantial changes in plant anatomical and physiological parameters (Barea et al. 2002; Högberg and Read 2006), while the effects of stress treatments for a well-adapted halophyte plant such as sea aster may be less pronounced compared to cultivated varieties. Sea aster is a specialist of saline or sodic grasslands and able to tolerate extreme water regimes (Shennan et al. 1987; Gray 1974). Stress effects may also be masked by genotypic and phenotypic heterogeneity of natural plant populations.

Plant growth parameters were, not surprisingly, good indicators of stress conditions, although the different trials revealed stress effects with differing sensitivity. Shoot dry weight (SDM), root dry weight (RDM), leaf area (LA) and plant height (PH) showed the highest variances in PC1 of experiment (I) and (II) However, neither SDM, RDM, nor longest leaf length (LL) were good indicators of stress in experiment (III) In case of a stress tolerant plant species such as sea aster, a well-timed measurement of phenotypical changes can indicate the stress effect more sensitive, than biomass data. Indeed number of leaves (LN) seems to be the most sensitive indicator for sea aster, provided the timing of measurement is appropriate. Stress conditions changed the number of leaves in a few days after the onset of stress. Similar phenotypical changes in leaves were detected in drought tolerant soybean plants by Ku et al. (2013), who found that plants responded to stress effects by leaf area changes without biomass loss. Hence these parameters may be sensitive indicators in certain species, although not generally applicable and should be optimised to each species and stress situation.

Suitability of drought or salinity stress symptoms characteristics, e.g., relative water content (RWC), wilting symptoms (WS), colour of leaves or dry leaf number (DL) were confirmed in the three experiments. It is of principal interest to find parameters that indicate stress conditions before the symptoms appear or biomass reduction is detectable.

Macro- and microelement concentrations of leaves were poor stress indicators in experiment II, only phosphorus

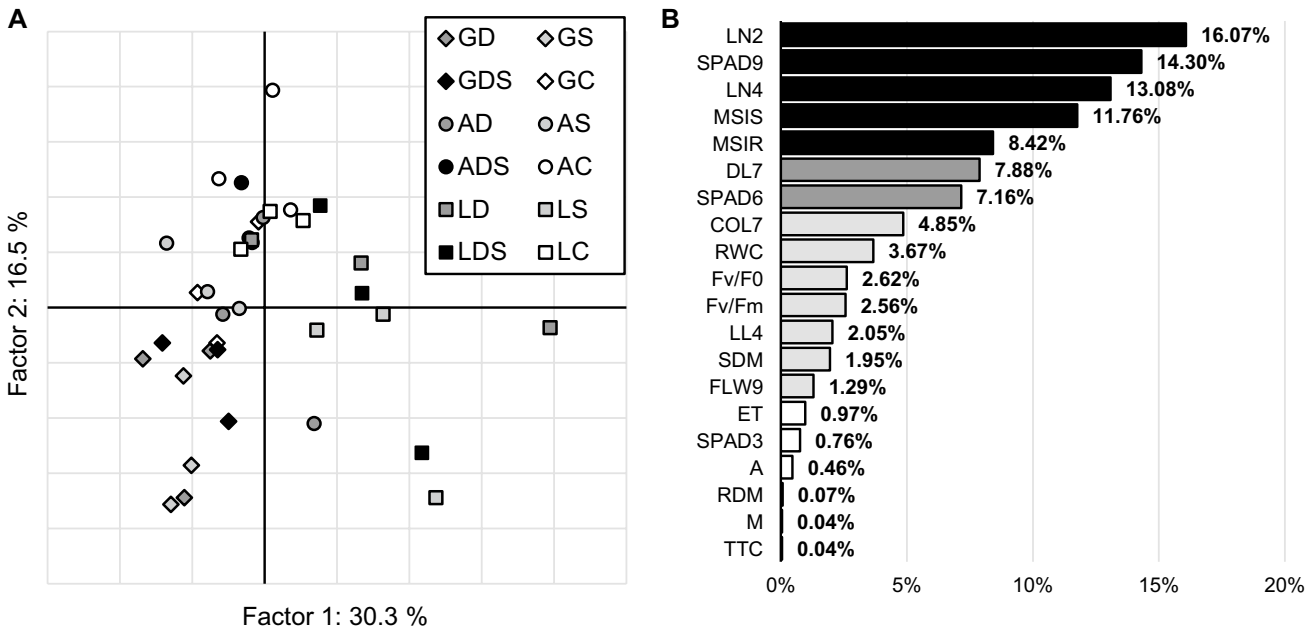

Fig. 3 The result of PCA in experiment III. **a** Projection of the cases on factor-plane (G/A/L: inoculation treatments *Glomus geosporum* strain/soil with indigenous AM fungi from artemisia steppe/soil with indigenous AM fungi from short grass pasture, C/D/S: control/drought/salt stressed plants), **b** the PC1 loading values of the 20 measured parameters (abbreviations of the traits are in Table 1)

and manganese concentration had a limited impact in PC1. Drought and salinity have a complex effect on plants: they can decrease transpiration rate, modify metabolic processes or the uptake of water and nutrients (Ahanger et al. 2014; Farooq et al. 2009; Hu and Schmidhalter 2005), while appropriate water supply can result in a dilution effect (James et al. 2005) with lower nutrient concentrations in control plants. These adverse effects can interfere, mitigate or extinguish the concentration changes.

The functioning of the photosynthetic apparatus can respond sensitively to environmental disturbances. Moderate drought typically impacts stomata, while metabolic and structural changes are provoked by severe or long-lasting drought (Jedmowski et al. 2013). Salt stress may also reduce the performance of the photosynthetic apparatus, mostly by the disorder of the electron transport system (Kalaji et al. 2016). Although chlorophyll content of plant leaves has been indicated as a good stress indicator (Li et al. 2006; Mehta et al. 2010; Ueda et al. 2003; Chaves et al. 2009), it could be only confirmed in experiment III (Fig. 3). Chlorophyll content may be diluted by a higher plant biomass similarly to macro- and micro-element concentrations of the shoot. A stress-induced decrease of biomass may, therefore, mitigate the parallel decline of chlorophyll content. Absence of growth retardation in experiment III hence may have contributed to expose the temporal decline in chlorophyll concentration during the duration of the experiment. No difference compared to the SPAD values of control plants was observed 3 weeks after the stress treatment started (SPAD3) which gradually had a higher impact after 6 and 9 weeks with 7% and 16% PC2 loading values, respectively (Fig. 3b).

F_v/F_m values were measured in all three experiment, but uniformly this parameter was not among the good stress indicators, in accordance with other studies with the same and other plant species (Andersone et al. 2012; Nakayama et al. 2007; Ohashi et al. 2006).

Physiological state of plants is well reflected by their relationship with their microsymbiont partners. A functional symbiosis requires a sensitive balance between the plant and mycorrhizal fungi or nodulating rhizobium bacteria thereby any changes in the plant will affect the symbionts and vice versa (Füzy et al. 2008a). N_2 fixation has been reported to decrease early in a drying soil (Serraj et al. 1990). Besides, drought may decrease nodule number and nodule biomass as well (Sinclair et al. 1988; Smith et al. 1988). Mycorrhizal colonization could be diminished (Al-Karaki et al. 2004) but also enhanced (Füzy et al. 2008a, b) by drought stress. Salinity stress may also influence the symbiotic relationship (Al-Karaki 2000; Kaya et al. 2009; Wu et al. 2010). Both soybean and sea aster are highly mycorrhiza dependent plants (Carvalho 2001; Howeler et al. 1987) which may have caused that mycorrhizal colonization parameters are only moderately strong stress indicators in experiment II and stress treatments had almost no effect on colonization rates in experiment III. Plants with weaker mycorrhiza dependency may respond to stress more sensitively through their symbiotic parameters.

Root capacitance measurement (EC) is a promising method in plant stress research, which is adequate for assessing root growth, length and surface area, as well as activity (Cseresnyés et al. 2013). Although an excellent monitoring tool, it is hardly adaptable for species with basal rosette leaves. The simple and non-intrusive record of both structural and functional characteristics makes EC measurement an excellent method for stress indication as it was shown in experiment (I) and (II) Likewise, membrane stability index (MSI) seemed to be a relevant stress indicator (Figs. 1a, 3a), which proved to be one of the key factors of variations especially when other obvious parameters did not show differences or could not be measured, as in experiment (III) Although not the most sensitive parameter, MSI is widely used to indicate stress conditions for several species and circumstances (Blum and Ebercon 1981; Bajji et al. 2002; Tripathy et al. 2000; Bouslama 1984). In contrast with numerous literature (Brini et al. 2009; Rahnama et al. 2010) the stomatal conductance measured in experiment I was not the most sensitive physological parameter either.

Time, cost of materials and especially the allocation of human resources are fundamental factors in the feasibility of experiments. It is, therefore, crucial to investigate plant parameters which are sensitive enough to minimize costs and labour with the highest benefits in meaningful data. Nonetheless, it is equally important not to underestimate the number of parameters which will properly characterize the often very slight or even highly complex phenological or functional changes a stress situation may cause. Stress responses may often lead to biomass differences and dilution of certain metabolites or nutrients in non-stressed individuals which can disturb the assessment of their parallel stress-related decrease in treated plants.

Consequently, it seems that chlorophyll content or functional parameters of the photosynthetic system are useful stress indicators preferably when plant biomass is constant among treatments. MSI and EC proved to be the most highly sensitive parameters that reliably detected even minute differences in plants as a consequence of stress conditions.

Author contribution statement AF designed drought stress experiment with soybean, determined root colonization parameters, read the relevant literature, carried out statistical analysis and wrote the paper. RK designed sea aster experiment with salt and drought stress, carried out root vitality test and membrane stability index measurements. IC and KR designed the salt stress experiment with wheat cultivars and carried out electrical measurements. IP measured photosynthetic parameters of plants and contributed to writing and language revision of the paper. TSK carried out ethylene production measurements. BK completed plant cultivation, plant growth monitoring, biomass measurements and observed stress symptoms. TT designed the plant experiments, carried out plant physiological investigations, read the relevant literature and participated in writing the paper. All authors read the manuscript and approved the submission.

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