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Intercropping induces physiological and morphological plasticity in oilseed rape and barley under drought stress

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Abstract: Intercropping is an agricultural practice that can improve crop yield due to better availability of resources, including water. There are few studies, however, addressing the physiological mechanisms behind this phenomenon. In this work oilseed rape (*Brassica napus* L.) and barley (*Hordeum vulgare* L.) were cultivated either as monocrop (MC) or intercrop (IC) under well-watered (WW) or drought stress (DS) conditions in a growth chamber. After eight weeks DS, the leaf relative water content was higher in the IC compared with the MC plants in both species and the DS-induced senescence of old leaves was considerably postponed in oilseed rape. Intercropped oilseed rape showed elevated levels of leaf photosynthesis rate, superior accumulation of organic osmolytes but higher water loss compared with the MC counterparts under DS conditions. In barley, less transpiration, an increased root : shoot ratio and osmolyte accumulation was observed in the IC compared with MC plants under DS conditions. The water use efficiency was higher in the IC compared to MC barley and the plants yield was higher in the IC than in the MC oilseed rape. Our data showed that intercropping is a reliable practice for cultivation of both species under arid and semi-arid regions or under rainfed conditions.

Key words: drought stress; intercropping; osmotic adjustment; photosynthesis rate;, transpiration: water use efficiency

This paper is part of PhD thesis of N.S under supervision of R.H; C.P was the advisor of this thesis.

Medsetev vzpodbuja fiziološko in morfološko prilagodljivost oljne ogrščice in ječmena v sušnem stresu

Izvleček: Medsetev je način kmetovanja, ki izboljšuje pridelek poljščin zaradi zaradi boljše dostopnosti virov, vključno z vodo. Malo je raziskav, ki bi se ukvarjale s fiziološkimi mehanizmi tega fenomena. V tej raziskavi sta bila v rastni komori gojena oljna ogrščica (*Brassica napus* L.) in ječmen (*Hordeum vulgare* L.) kot monokultura (MC) ali kot mešan posevek (IC) v razmerah dobre preskrbe z vodo (WW) ali v razmerah sušnega stresa (DS). Po osmih tednih rasti v sušnem stresu je bila relativna vsebnost vode pri obeh vrstah večja pri medsetvi kot v monokulturi, pri oljni ogrščici je bilo odmiranje starih listov v razmerah sušnega stresa znatno kasnejše. Oljna ogrščica je imela v medsetvi v razmerah sušnega stresa večjo fotosintezo, večje kopičenje osmotikov, a večjo izgubo vode v primerjavi z gojenjem v monokulturi. Pri ječmenu je bila pri medsetvi v razmerah sušnega stresa manjša transpiracija, povečano razmerje korenina : poganjek, povečana akumulacija osmotikov v primerjavi z rastjo v monokulturi. Učinkovitost izrabe vode je bila pri ječmenu večja v medsetvi kot v monokulturi, v medsetvi je bil večji tudi pridelek oljne ogrščice. Ti podatki kažejo, da je medsetev primeren način gojenja obeh vrst v sušnih in polsušnih območjih v razmerah preskrbe z vodo z deževjem.

Ključne besede: sušni stres; medsetev; osmotska prilagoditev; velikost fotosinteze; transpiracija; učinkovitost izrabe vode

Članek je del disertacije N.S. pod mentorstvom R.H. in svetovanjem C.P.

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1 INTRODUCTION

Drought stress is one of the most important environmental constrains limiting plants production worldwide (Tardieu et al., 2018). Photosynthesis, the key process responsible for growth and dry matter production of plants, decreases under water stress through both stomatal and non-stomatal limitations (Zhou et al., 2013). Nonstomatal factors such as decreased leaf expansion and photosynthetic pigments concentration, leaf senescence and reduced electron transport activities, in combination with stomatal factors, reduce the overall photosynthetic performance of plants under drought stress (Chaves et al., 2009).

Since the CO_2 assimilation is decreased simultaneously with transpiration under water stress, the efficiency of plants for photosynthesis or biomass production at the expense of a given rate of water loss, i.e. water use efficiency (WUE), is an important parameter for plants drought tolerance (Tambussi et al., 2007).

Plants adopt various strategies for confronting drought stress and survive under these conditions. The increased production of low molecular weight organic osmolytes such as free amino acids particularly proline and soluble carbohydrates is crucial for the regulation of cell water content under extreme osmotic environment (Singh et al., 2015). By decreasing the osmotic potential in the cytoplasm, these osmoprotectants help plants to prevent cell dehydration. Moreover, these organic osmolytes contribute to mitigate damage caused by reactive oxygen species (ROS), to prevent membrane injury and to stabilize proteins and enzymes (Krasensky and Jonak, 2012).

Intercropping is establishing two or more crop species together at same field in the same time. Under intercropping conditions, both negative interaction (competition) and positive interaction (facilitation) can occur simultaneously (Brooker et al., 2015). However, by increasing facilitation and decreasing competition between crops, intercropping systems can use environmental resources more effectively. In fact, higher yield has been repeatedly recorded in many intercropping systems compared to monocultures (Martin-Guay et al., 2018). There are evidences showing that biomass and water use efficiency (WUE) of intercropping systems under drought stress are usually greater than that of monocultures (Daneshnia et al., 2015; Chimonyo et al., 2016). There are, however, studies that showed intercropping systems did not increase obviously WUE (Grema and Hess, 1994; Shackel and Hall, 1984), or sometime reduced it (Rees, 1986; Singh et al., 1988; Gao et al., 2009).

Belowground interactions in the ecological and agricultural systems are not restricted to the competition or facilitation mechanisms for nutrient acquisition (Mommer et al., 2016). Increasing evidences obtained from plants co-cultured under laboratory conditions showed considerable influence of both interspecific and conspecific interactions on plants development, metabolism and defense (Schmid et al., 2013; Chen et al., 2018). Almost all of these effects are independent from competition or complementary usage of resources (Semchenko et al., 2014; Kong et al., 2018).

Almost all of previous works on the effect of cropping pattern on plants drought resistance have been undertaken under field conditions with little attention paid to explore the mechanisms behind the improvement of drought tolerance in the intercrop systems. In order to explore the physiological and biochemical effects of belowground root interactions, we cultivated oilseed rape plants and barley under well-watered or drought stress conditions either as monocrop or intercrop and analyzed plants for water content and osmotic parameters. Our working hypothesis is that, the interspecific interactions in the intercrop system may trigger some biochemical and physiological modifications in the co-cultured plants that influence their response to drought.

2 MATERIALS AND METHODS

2.1 PLANTS CULTURE AND TREATMENTS

Seeds of oilseed rape (*Brassica napus* 'Opera') and barley (*Hordeum vulgare* 'Makoui') plants were provided by the Seed and Plant Improvement Institute (Karaj, Iran) and Dryland Agricultural Research Institute (DARI) (Maragheh, Iran), respectively. The seeds were surface sterilised using commercial bleach and germinated in the dark on perlite. After germination, the seedlings were transferred to the light. The 10-day old young oilseed rape seedlings were precultured in the 50 % Hoagland nutrient solution for two weeks before starting the experiment.

Twenty five-day-old oilseed rape together with one-week-old barley seedlings were transferred to 0.8 l plastic pots filled with perlite and cultivated either as monocrop (MC) or intercrop (IC). Since the biomass and leaf area of one barley seedling was a quarter of oilseed rape, 4 barley plants were cultivated with one oilseed rape in the IC pots. In the MC pots either two oilseed rape or 8 barley plants were cultivated.

Two weeks after starting MC/IC treatments, two watering regimes including well-watered (WW) and drought stress (DS) were assigned randomly to the pots. The WW plants were continued to be irrigated to 100 % field capacity (FC) while watering was omitted from DS pots until they reached the 30 % FC. This was achieved one week after starting the DS treatment.

Everyday throughout the experiment, after weighing, the pots were irrigated with nutrient solution or water as interval. Control and water-stressed plants received the same amount of nutrient solution and the respective FC was achieved by different volumes of water used for irrigation. Water consumption (~water loss; the amount of water needed for adjustment of pots to the respective FC) was recorded daily.

2.2 HARVEST

The plants were harvested eight weeks after reaching the 30 % FC (10 weeks after starting MC/IC treatments). The roots were separated from perlite and washed with distilled water and blotted dry on filter paper. After determination of fresh mass (FM), leaf and root samples were oven-dried at 70 °C for 48 h, and dry mass (DM) was determined. Because of almost complete intertwining the roots in the IC pots under WW conditions, the root mass could not be determined for two species separately.

2.3 MEASUREMENT OF SPAD AND LEAF CHL CONCENTRATION

Leaf greenness was measured daily as the Spectral Plant Analysis Diagnostic (SPAD) index in the second youngest, fully expanded leaf (young leaf) and in the second oldest leaf (old leaf) using a chlorophyll-meter (Minolta, 502). Leaf Chl concentration in the young and old leaves was spectrophotometrically determined after extraction in 70 % aceton for 24 h in the dark at 4 °C (Lichtenthaler and Wellburn, 1985).

2.4 DETERMINATION OF GAS EXCHANGE PARAMETERS AND WATER USE EFFICIENCY

Net CO_2 assimilation rate, transpiration and stomatal conductance to water vapour were measured in the attached young and old leaves with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00 a.m at a photosynthetic photon flux density of 350 μmol m^{-2} s^{-1}.

The instantaneous water use efficiency (iWUE) (μmol $mmol^{-1}$) was defined at leaf scale as the net photosynthesis rate divided by the water transpired in the same time period:

$$iWUE = \frac{Photosynthesis\ rate}{Transpiration\ rate}$$

The biomass WUE (bWUE) (g kg^{-1}) was defined at whole plant scale as the ratio of biomass produced to the rate of water consumed (Tambussi et al., 2007):

$$bWUE = \frac{Shoot\ DM}{Total\ water\ consumption}$$

2.5 MEASUREMENT OF RELATIVE WATER CONTENT AND OSMOTIC POTENTIAL

Relative water content (RWC %) was measured in the leaves harvested 1 h after starting the light period and calculated according to the following equation:

$$RWC\ (\%) = \frac{FM - DM}{TM - DM} \times 100$$

For determination of turgid mass (TM), leaf disks (5 mm diameter) were submerged for 5 h in distilled water, thereafter, they were blotted dry gently on a paper towel and weighed.

Osmotic potential was determined in the leaf and root samples harvested at 1 h after the lights were turned on in the growth chamber. Samples were homogenized in prechilled mortar and pestle and centrifuged at 4000 g for 20 min at 4 °C. The osmotic pressure of the samples was measured by an osmometer (Heman Roebling Messtechnik, Germany), and the miliosmol data were recalculated to MPa.

2.6 DETERMINATIONS OF BIOCHEMICALS

For determination of soluble carbohydrates, leaf and root samples were homogenized in ethanol at 4 °C. After centrifugation at 12,000 *g* for 15 min, an aliquot of the supernatant was mixed with anthrone-sulfuric acid reagent and incubated for 10 min at 100 °C. After cooling, the absorbance was determined at 625 nm (Yemm and Willis, 1954). Glucose (Merck) was used to construct a standard curve. Total soluble proteins were determined using a commercial reagent (Bradford reagent, Sigma) and bovine albumin serum as standard. Proline was extracted and determined by the method of Bates et al. (1973). Leaf tissues were homogenized with 3 % sulfosalicylic acid and the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve. Content of total free a-amino acids was assayed using a ninhydrin colorimetric method (Yemm and Cocking, 1955). Glycine was used for standard curve.

2.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was a complete randomised block with four independent pots as four replications. Pairwise comparison of means was performed by the Tukey's test ($p < 0.05$) using Sigma stat (3.02). To assign different physiological parameters to distinct groups, principal component analysis (PCA) was conducted using Minitab 18.

3 RESULTS AND DISCUSSION

3.1 EFFECT OF DS AND IC ON THE BIOMASS AND LEAF AREA

Drought stress (DS) decreased shoot biomass and leaf area of both species (Fig. 1). However, the effects of DS on the shoot biomass and leaf area under MC conditions were not significant in the barley and oilseed rape plants, respectively (Fig. 1). Leaf growth is accomplished through cell division and cell expansion which are both affected by water deficit (Koch et al., 2019). Cell expansion is one of the most drought-sensitive physiological processes because of its dependence on the turgor pressure. Impaired cell division and expansion results in reduced plant height, leaf area and ultimately growth reduction of plant under drought (Skirycz and Inze, 2010). Under long term water deficit as in our work, biomass of plants is also decreased due to the reduced CO_2 assimilation rate (Tardieu and Granier, 2011).

Similar to the shoot growth parameters, root biomass decreased under DS conditions in both species cultivated in the MC pots. The responses of root growth and elongation to drought largely depend on the plant species, the genotype and the severity of drought stress (Sánchez-Blanco et al., 2014). Under mild drought stress root : shoot ratio may increase as the result of a preferential allocation of photosynthates to the roots allowing better water capture as an adaptation to drought (Faroog et al., 2009). Under severe drought, in contrast, root biomass and length is decreased likely because the limited photosynthesis reduces the sucrose export to the roots and ultimately inhibits root growth (Lemoine et al., 2013). Here in our work, root : shoot ratio was not modified by DS in oilseed rape while decreased from 1.0 under WW to 0.71 under DS conditions in barley (Fig. 1).

Intercropped (IC) oilseed rape plants showed higher shoot biomass than the monocropped (MC) counterparts under both well-watered (WW) and DS conditions. Barley, in contrast, produced less shoot biomass when cultivated in the IC pots irrespective the watering regime (Fig. 1). The leaf area also increased under IC conditions in oilseed rape under WW conditions, while this parameter decreased in barley both under WW and DS conditions (Fig. 1). The improvement of shoot growth under intercropping conditions in oilseed rape, but its depression in barley that was observed independent from watering treatments will be discussed below.

Root biomass was not influenced by the IC treatment in oilseed rape, while increased in barley under DS conditions (Fig. 1). The improvement of root biomass in IC barley grown under DS conditions contrasted with the effect of intercropping on shoot biomass and leaf area in this species. This led to an increase in root : shoot ratio from 0.71 in the MC to 3.12 in the IC barley plants, while this ratio was not influenced by intercropping in oilseed rape plants. The effect of IC on WW plants could not be detected because of lacking individual data for each species (see M & M). The total root biomass of plants in the IC pots (1.90 ± 0.22) was significantly higher ($p < 0.05$) than the sum of two MC pots (1.29 ± 0.37) (data not shown).

3.2 EFFECT OF DS AND IC ON THE CHL CONCENTRATION, PHOTOSYNTHESIS AND TRANSPIRATION RATES

The Chl a + b concentration was not influenced by DS in barley but decreased in the old leaves of oilseed rape plants (Table 1). Reduction of Chl under DS is likely the results of higher rates of degradation mainly due to the elevated levels of ROS under these conditions (Noctor et al., 2014). Loss of the balance between the production and scavenging of ROS induces oxidative stress and the accumulated ROS damages proteins, pigments, membrane lipids and other cellular components (Cruz de Carvalho, 2008).

The photosynthesis and transpiration rates decreased significantly by DS in the old leaves of both species and in the young leaves of oilseed rape (Table 1). Reduction of transpiration through stomatal control of water losses has been identified as an early event in plant response to water deficit leading in turn to limitation of

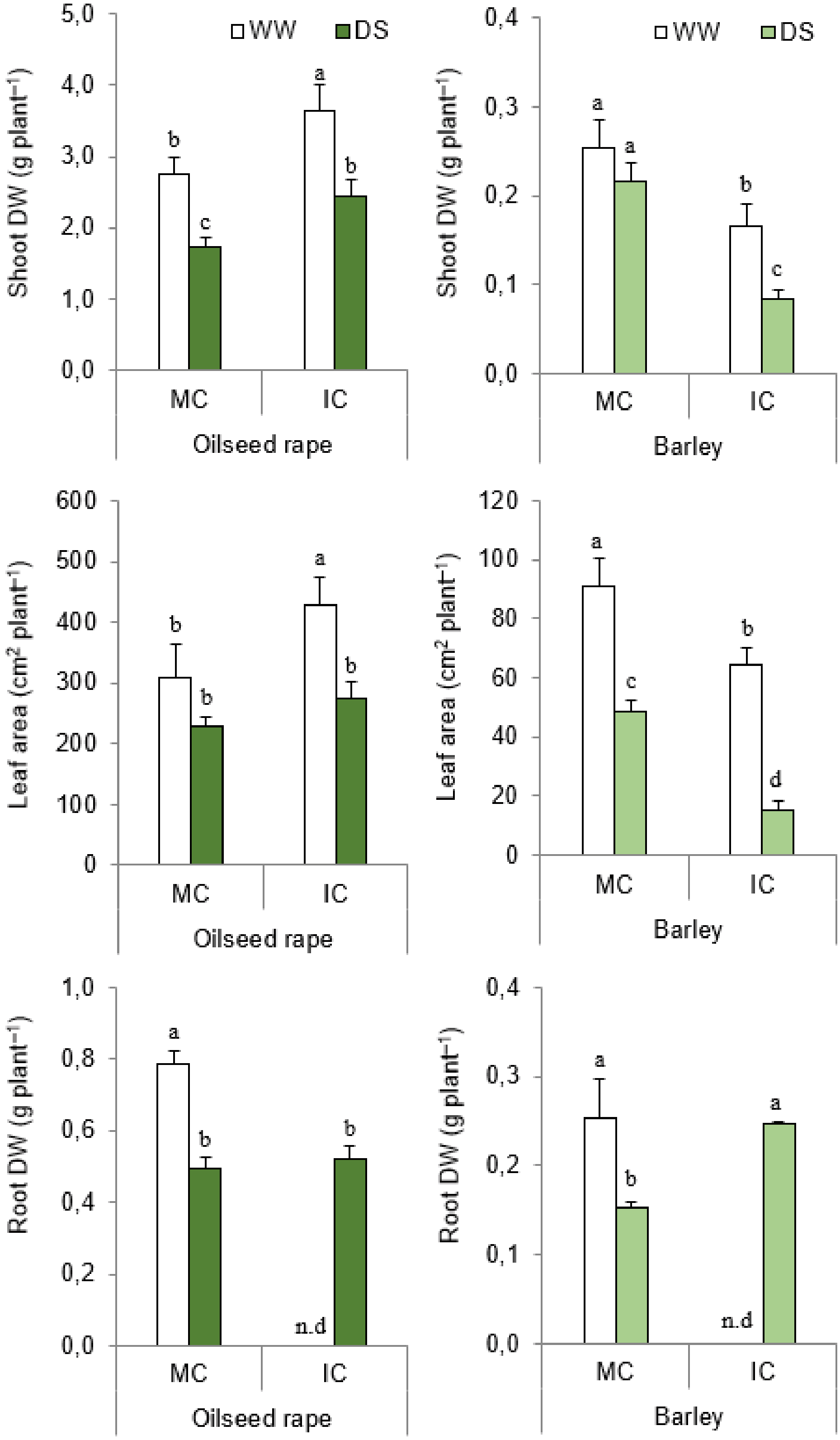

Figure 1: Shoot and root dry biomass and leaf area in oilseed rape and barley cultivated either as monocrop (MC) or intercrop (IC) under well-watered (WW) or drought stress (DS) conditions for eight weeks. Bars indicated by the different letters are significantly different ($p < 0.05$)

CO_2 diffusion into the leaves (Zhou et al., 2013). Since the activity of the photosynthetic electron transport chain is finely tuned to the availability of CO_2 in the chloroplast, restricted CO_2 availability could lead to increased susceptibility to damage to photosynthetic apparatus (Chaves et al., 2002). In addition, reduction in photosynthesis arises by impaired activities of Calvin cycle enzymes and a decline in Rubisco activity (Chaves et al., 2009).

Intercropping did not affect Chl a + b concentration in the young leaves of oilseed rape plants, but increased it in the old leaves of both WW and DS plants. In barley, concentration of Chl a + b was not influenced in either of the leaves under WW or DS conditions (Table 1). In oilseed rape, IC treatment increased photosynthesis rate in the young leaves of DS plants and in the old leaves of both WW and DS plants. In barley, in contrast, leaf photosynthesis rate was not influenced by intercropping (Table 1). Transpiration rate increased by IC treatment in oilseed rape that was significant for the old leaves, while decreased in the young leaves of barley (Table 1).

3.3 EFFECT OF IC ON THE PHENOTYPIC PLASTICITY OF BOTH SPECIES UNDER DS

The cropping pattern influenced plants response to DS differently depending on species. In the DS oilseed rape, IC conditions resulted in a slight increase in the leaf area (Fig. 1), and photosynthesis and transpiration rates per surface area (Table 1). Despite the putatively higher water loss at whole plant level under IC conditions, this strategy may enable this species to keep higher ability for biomass production and synthesis of osmolytes (see below) compared with the MC counterparts. In barley, in contrast, reduction of leaf area and transpiration rate per surface area most likely led to lower water loss at whole plant level accompanied by an increased root biomass and higher root : shoot ratio. Such phenotypic plasticity in response to DS in barley that was observed only under IC conditions may enable this species to capture efficiently water from the dry substrate. Root growth and density, proliferation and size are key responses of plants to drought stress (Farooq et al., 2009). It is well plausible that the belowground root interactions in the IC pots mediate some modifications in the phytohormone balances in plants. In our barely plants, reduction of shoot growth and an increase in the root : shoot ratio under DS conditions are the well-known responses of plants to abscisic acid (Mc Adam et al., 2016). Modification in the levels of phytohormones through root interactions with the neighbor plants has been observed in tobacco (Chen et al., 2018). From an ecological point of view, the ability of plants to plastically adjust to environment play important role in the function of mixed cropping systems (Zhu, 2015).

3.4 EFFECT OF DS AND IC ON WATER STATUS OF THE YOUNG AN OLD LEAVES

Drought stress expectedly decreased RWC in the young and old leaves of both species (Fig. 2). Intercropping did not influence the leaf RWC in the WW plants while significantly increased this parameter in the young

Table 1: Concentrations of chlorophyll (Chl) a + b , photosynthesis and transpiration rates in the young and old leaves of oilseed rape and barley cultivated either as monocrop (MC) or intercrop (IC) under well-watered or drought stress conditions for eight weeks. Data of each column indicated by the different letters are significantly different ($p < 0.05$)

Oilseed rape Young leaf		Chl a + b (mg g^{-1} FM)		Photosynthesis (μmol m^{-1} s^{-1})		Transpiration rate (mmol m^{-1} s^{-1})	
		Old leaf	Young leaf	Old leaf	Young leaf	Old leaf	
Well-watered	MC	6.45 ± 0.85^{a}	2.17 ± 0.32^{c}	4.62 ± 0.12^{ab}	2.43 ± 0.39^{b}	1.41 ± 0.17^{a}	1.22 ± 0.20^{a}
	IC	7.08 ± 0.61^{a}	6.02 ± 0.74^{a}	5.35 ± 0.34^{a}	3.46 ± 0.42^{a}	1.58 ± 0.43^{a}	1.39 ± 0.14^{a}
Drought stress	MC	5.85 ± 0.87^{a}	1.67 ± 0.67^{c}	2.84 ± 0.28^{c}	1.48 ± 0.29^{c}	0.75 ± 0.15^{b}	0.30 ± 0.02^{c}
	IC	6.04 ± 0.37^{a}	4.12 ± 0.39^{b}	4.22 ± 0.60^{b}	2.71 ± 0.19^{b}	1.18 ± 0.08^{ab}	0.67 ± 0.01^{b}
Barley Young leaf		Chl a + b (mg g^{-1} FM)		Photosynthesis (μmol m^{-1} s^{-1})		Transpiration rate (mmol m^{-1} s^{-1})	
		Old leaf	Young leaf	Old leaf	Young leaf	Old leaf	
Well-watered	MC	5.63 ± 0.65^{a}	3.45 ± 0.27^{a}	5.43 ± 0.72^{a}	3.97 ± 0.68^{a}	1.38 ± 0.07^{a}	0.85 ± 0.10^{a}
	IC	5.45 ± 0.31^{a}	3.04 ± 0.36^{a}	5.46 ± 0.44^{a}	3.64 ± 0.54^{ab}	1.30 ± 0.21^{a}	0.73 ± 0.19^{a}
Drought stress	MC	5.21 ± 0.78^{a}	3.26 ± 0.42^{a}	4.36 ± 0.17^{a}	2.71 ± 0.12^{b}	1.30 ± 0.08^{a}	0.35 ± 0.02^{b}
	IC	5.51 ± 0.64^{a}	3.00 ± 0.32^{a}	3.88 ± 0.69^{a}	2.94 ± 0.62^{ab}	0.66 ± 0.15^{b}	0.35 ± 0.05^{b}

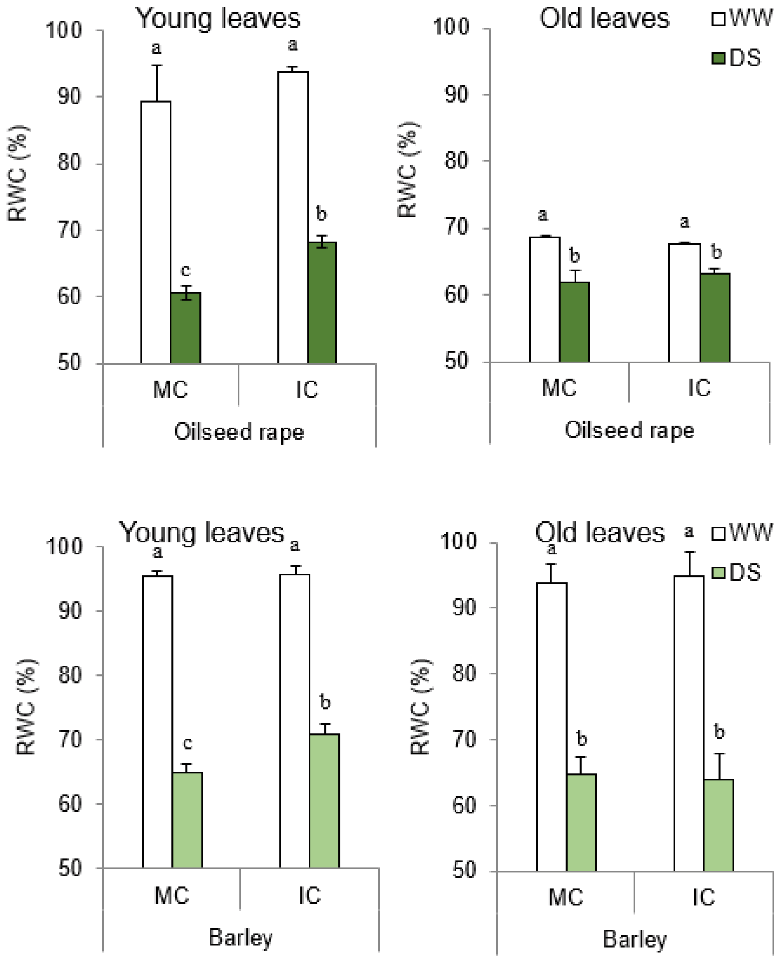

Figure 2: Relative water content (RWC) in the young and old leaves of oilseed rape and barley cultivated either as monocrop (MC) or intercrop (IC) under well-watered (WW) or drought stress (DS) conditions for eight weeks. Bars indicated by the different letters are significantly different ($p < 0.05$)

leaves of both species under DS conditions (Fig. 2). The leaf RWC is a reliable parameter to evaluate the water status of plants that reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan and Ciulca, 2011). The improvement of RWC in the young leaves of both species upon intercropping in this work is an indication of an interspecific interaction occurred only under water deficit conditions being independent from the effect of IC on biomass production.

3.5 EFFECT OF DS AND IC ON THE WUE AND WATER CONSUMPTION

Instant WUE (iWUE) increased under DS conditions in the old leaves of both species that was observed for both MC and IC plants (Fig. 3). Significant effect of IC on iWUE was observed in the young leaves of barley under DS conditions (Fig. 3). Drought stress increased the biomass WUE (bWUE) too (Fig. 3). This parameter differed also significantly among three culture pots; the lowest bWUE was observed in the MC barley pots both under WW and DS conditions (Fig. 3). Increases in WUE are commonly stated as a response of plants to moderate to severe water deficiency (Tambussi et al., 2007). There are evidences showing that the WUE of intercropping systems are usually greater than that of monoculture (Daneshnia et al., 2015; Chimonyo et al., 2016). There are, however, studies that showed intercropping systems did not increase obviously WUE (Grema and Hess, 1994; Shackel and Hall, 1984), or sometime reduced it (Rees, 1986; Singh et al., 1988; Gao et al., 2009). Here in our work, IC pots have higher bWUE than the MC barley pots both under WW and DS conditions. In oilseed rape, intercropping did not influence bWUE under DS conditions but decreased it under WW conditions (Fig. 3).

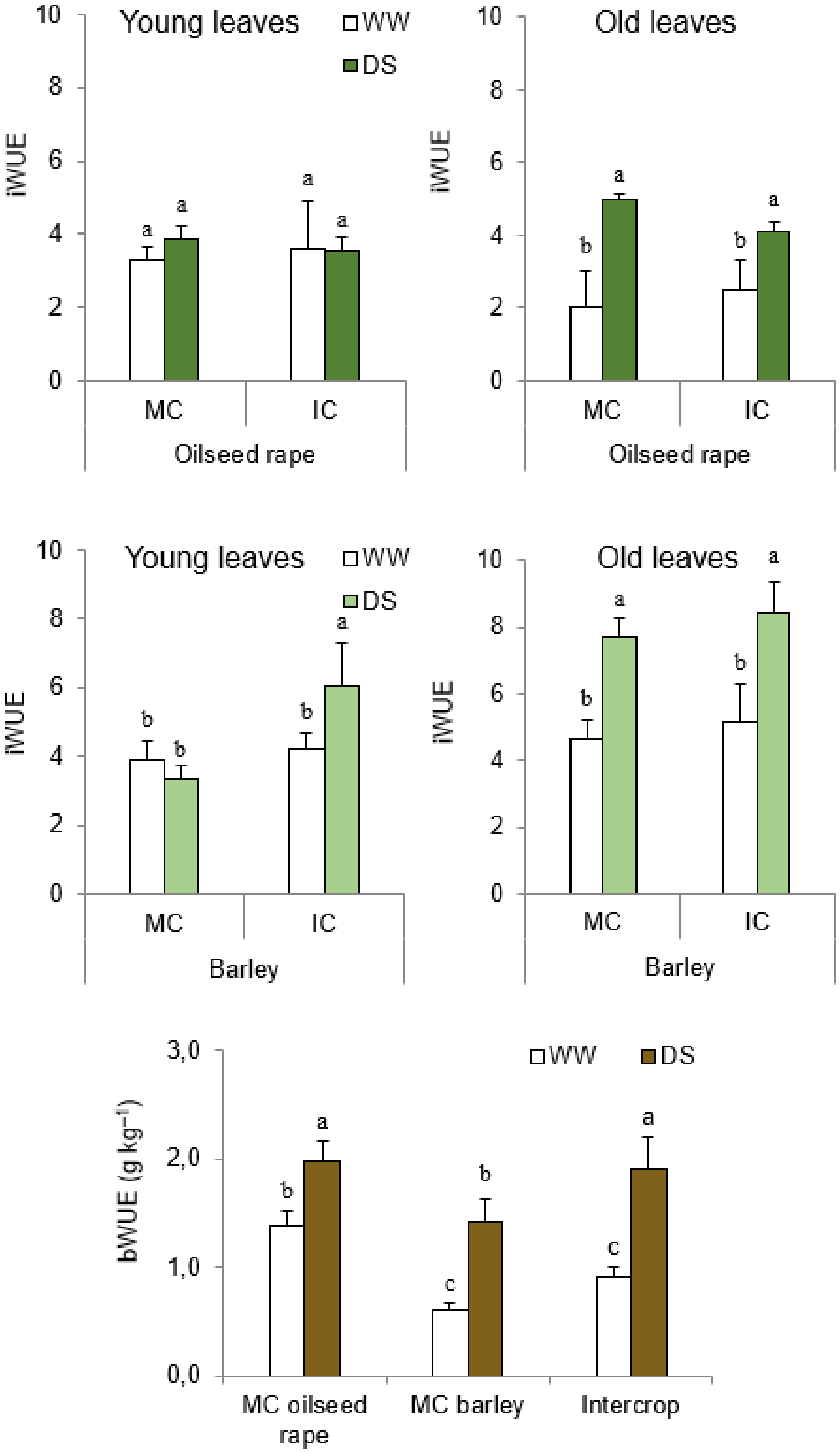

Figure 3: Instant water use efficiency (iWUE) in the young and old leaves of oilseed rape and barley and biomass water use efficiency (bWUE) in the monocrop (MC) or intercrop (IC) pots after eight weeks cultivation under well-watered (WW) and drought stress (DS) conditions. Bars within each culture mode indicated by the different letters are significantly different ($p < 0.05$)

Daily water consumption gradually increased during the two months experiment in both MC and IC pots under WW conditions (Fig. 4). Difference between MC and IC pots were obvious from 30 days after intercrop onward, and at the end of experiment, daily water consumption in the IC pots was considerably higher than that in MC pots (Fig. 4). Under DS conditions, the water consumption sharply decreased subsequent to omitting watering and remained lower throughout the experiment. Daily water consumption was consistently higher

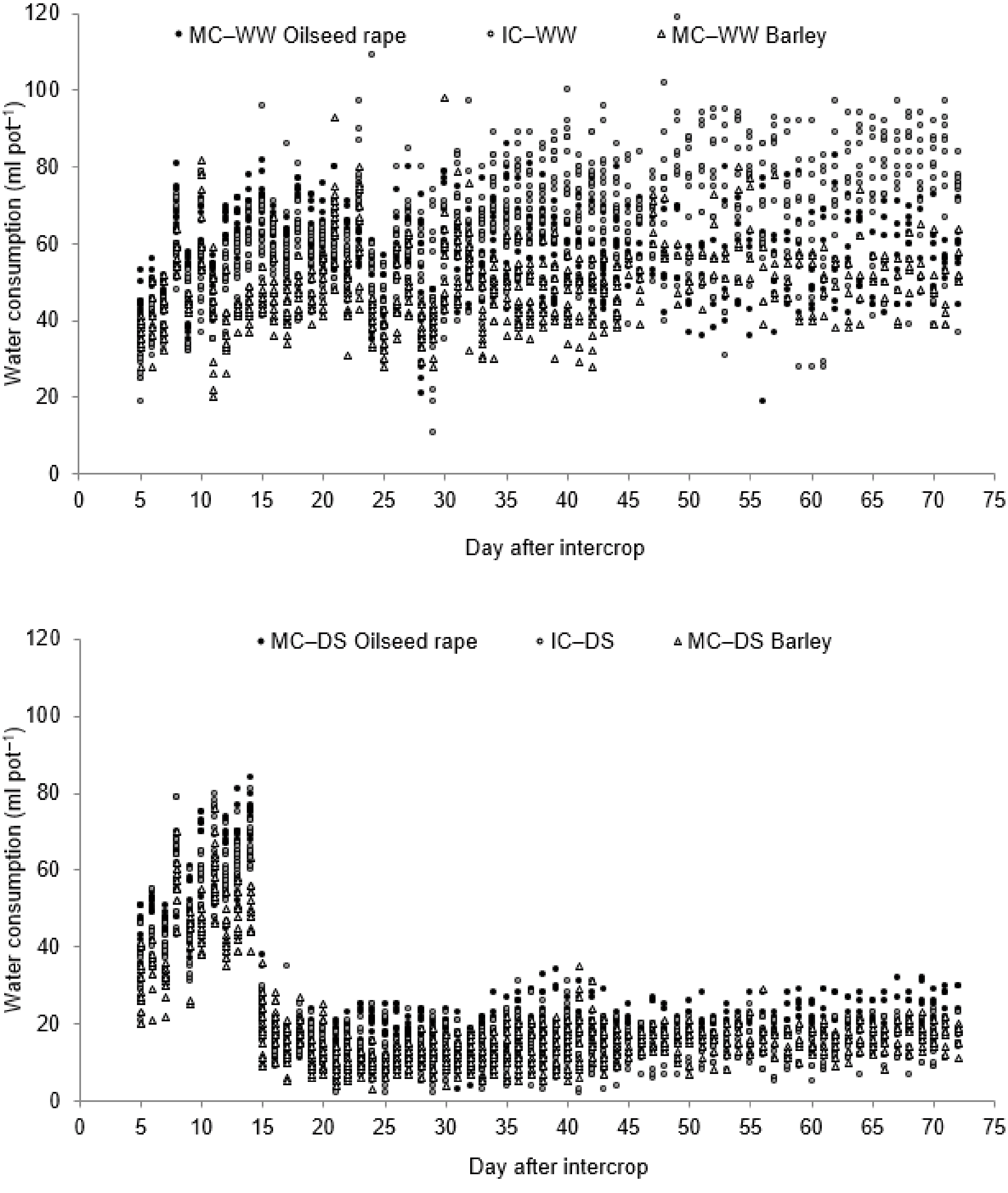

Figure 4: Daily water consumption of the monocrop (MC) pots of oilseed rape and barley and of the intercrop (IC) pots under well-watered (WW) (above) or drought stress (DS) (below) conditions for eight weeks

in the MC oilseed rape pots compared with IC and MC barley pots (Fig. 4).

3.6 EFFECT OF DS AND IC ON THE OSMOTIC HOMEOSTASIS OF LEAVES AND ROOTS

Leaf osmotic potential decreased under DS conditions in both species. Effect of DS on the root osmotic potential, however, was significant only in oilseed rape (Table 2). Leaf concentration of organic osmolytes increased under DS conditions in oilseed rape. Significant effect of DS, however, was observed for proline in the young leaves and for soluble sugars in the old leaves while free amino acids contributed equally to the osmolytes concentration in the old and young leaves of this species (Table 2). In barley leaves, soluble sugars did not respond to the treatments. The effect of DS on the proline concentration was not significant but the free amino acids increased in the young leaves of this species in response to DS. In the roots, proline and soluble sugars accumulated in both species while free amino acids did not responds to DS in none of species (Table 2).

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes including soluble sugars, free amino acids, proline and glycinebetaine (Singh et al., 2015). These osmolytes protect plants through contribution to osmotic adjustment, detoxification of ROS, and the stabilization of membranes, native structures of enzymes and proteins (Verbruggen and Hermans, 2008). Oilseed rape responded more to the DS than barley regarding the accumulation of osmolytes in the leaves. This may allow this species to have higher RWC despite higher transpiration that help also to produce biomass under DS conditions. Of particular importance was the proline accumulation particularly in the young leaves of oilseed rape that was much higher than that in barley. Proline accumulation is caused by a combination of increased biosynthesis and slow oxidation in mitochondria (Parida et al., 2008) and play important roles including stabilization of macromolecules, ROS scavenging, a sink for excess reductant

Table 2: Osmotic potential and concentrations of proline, free amino acids and soluble sugars in the young and old leaves and roots of oilseed rape and barley cultivated either as monocrop (MC) or intercrop (IC) under well-watered or drought stress conditions for eight weeks. Data of each column indicated by the different letters are significantly different ($p<0.05$)

Oilseed rape		Young leaf	Old leaf	Roots	Young leaf	Old leaf	Roots
		Osmotic potential (−MPa)			Proline (µmol g^{-1} FM)		
Well-watered	MC	0.564 ± 0.030^{b}	0.469 ± 0.079^{b}	0.101 ± 0.013^{b}	0.72 ± 0.18^{c}	0.25 ± 0.06^{b}	0.40 ± 0.06^{b}
	IC	0.524 ± 0.015^{b}	0.511 ± 0.003^{b}	0.118 ± 0.009^{b}	0.76 ± 0.09^{c}	0.35 ± 0.04^{b}	0.27 ± 0.06^{b}
Drought stress	MC	0.904 ± 0.039^{a}	0.832 ± 0.003^{a}	0.196 ± 0.011^{a}	3.10 ± 0.69^{b}	0.67 ± 0.22^{b}	1.50 ± 0.16^{a}
	IC	0.962 ± 0.032^{a}	0.909 ± 0.049^{a}	0.224 ± 0.031^{a}	5.47 ± 0.61^{a}	1.14 ± 0.35^{a}	1.63 ± 0.63^{a}
		Free amino acids (µmol g^{-1} FW)			Soluble sugars (mg g^{-1} FM)		
Well-watered	MC	5.22 ± 0.11^{c}	3.04 ± 0.78^{d}	4.09 ± 1.28^{b}	29.43 ± 3.37^{b}	25.91 ± 5.27^{b}	0.63 ± 0.07^{c}
	IC	7.48 ± 0.69^{b}	7.28 ± 1.14^{b}	4.78 ± 1.45^{b}	28.87 ± 0.99^{b}	24.88 ± 6.11^{b}	1.42 ± 0.08^{c}
Drought stress	MC	8.67 ± 1.69^{b}	5.23 ± 0.57^{c}	5.90 ± 0.97^{b}	35.91 ± 3.12^{ab}	48.79 ± 4.17^{a}	2.76 ± 0.28^{b}
	IC	17.0 ± 2.53^{a}	11.4 ± 1.09^{a}	9.09 ± 0.48^{a}	47.03 ± 11.7^{a}	49.89 ± 10.8^{a}	4.07 ± 0.78^{a}
Barley		Young leaf	Old leaf	Roots	Young leaf	Old leaf	Roots
		Osmotic potential (−MPa)			Proline (µmol g^{-1} FM)		
Well-watered	MC	0.588 ± 0.014^{b}	0.543 ± 0.048^{b}	0.122 ± 0.008^{b}	0.25 ± 0.04^{a}	0.15 ± 0.02^{b}	0.22 ± 0.03^{c}
	IC	0.521 ± 0.057^{b}	0.511 ± 0.033^{b}	0.131 ± 0.008^{b}	0.24 ± 0.07^{a}	0.16 ± 0.04^{b}	0.23 ± 0.05^{c}
Drought stress	MC	0.856 ± 0.177^{a}	0.898 ± 0.071^{a}	0.141 ± 0.005^{b}	0.36 ± 0.04^{a}	0.31 ± 0.07^{ab}	0.66 ± 0.00^{b}
	IC	0.993 ± 0.022^{a}	0.985 ± 0.032^{a}	0.258 ± 0.028^{a}	0.41 ± 0.15^{a}	0.39 ± 0.15^{a}	1.48 ± 0.14^{a}
		Free amino acids (µmol g^{-1} FW)			Soluble sugars (mg g^{-1} FM)		
Well-watered	MC	2.79 ± 0.80^{b}	4.07 ± 0.07^{a}	1.82 ± 0.37^{b}	7.22 ± 1.52^{a}	7.25 ± 2.25^{a}	0.72 ± 0.03^{c}
	IC	2.85 ± 0.58^{b}	4.46 ± 0.58^{a}	1.63 ± 0.21^{b}	7.16 ± 1.29^{a}	7.98 ± 2.96^{a}	0.94 ± 0.09^{c}
Drought stress	MC	5.49 ± 1.53^{a}	4.28 ± 0.45^{a}	2.03 ± 0.13^{b}	7.93 ± 1.88^{a}	7.32 ± 2.31^{a}	1.99 ± 0.27^{b}
	IC	4.94 ± 0.43^{a}	4.45 ± 0.40^{a}	2.86 ± 0.24^{a}	7.58 ± 2.96^{a}	11.0 ± 2.51^{a}	3.94 ± 0.15^{a}

and a source for carbon and nitrogen for use after relief of water deficit (Verbruggen and Hermans, 2008; Szabados and Savoure, 2009).

Intercropping did not influence the leaf or root osmotic potential in oilseed rape but decreased it in the roots of barley grown under DS conditions (Table 2). In the oilseed rape plants grown under DS conditions, IC plants showed higher concentration of proline and free amino acids in the leaves and higher free amino acids and soluble sugars in the roots compared to the MC plants. In the WW oilseed rape plants, only the leaf concentration of free amino acids was altered by the IC treatment. In barley, leaves did not respond to the IC treatment either in the DS or WW plants, while the roots accumulated all three osmolytes under DS conditions and proline and soluble sugars under WW conditions (Table 2).

The mechanisms behind the influence of the cropping pattern on the osmolyte accumulation are obscure. Intensification of water deficit following an increased competition for water and a faster depletion from the substrate in the IC pots could not be the mechanism for higher osmolytes accumulation. Indeed, the severity of DS could not be affected by cropping pattern because of daily irrigation up to the desired FC in our experiment. In addition, the total water consumption was rather lower in the IC pots compared to the MC oilseed rape (Fig. 1). A modification in the metabolism of plants under the effects of belowground root interactions is not restricted to the influence on the concentrations of organic osmolytes observed in this work and seems to be rather common in intercropping systems. In a proteomics analysis in the millet/peanut intercrop system, the expression of several proteins that are mainly involved in carbon and nitrogen metabolism are upregulated by interspecific root interactions (Zou et al., 2019)._

To evaluate the relevance of the different osmotic adjustment parameters in the plants responses to the applied treatments, data were subjected to PCA (Fig. 5). The result showed that the photosynthesis and transpiration rates and the RWC were clustered with biomass data and, thus, were likely the most important parameters determining the plants response to the applied treatments (Fig. 5). Contrastingly, the osmotic adjustment parameters were separately clustered from the biomass data in both species. This was unexpected because the osmolytes contribute undoubtedly to sustaining leaf turgor required for photosynthesis and growth. Nevertheless, results of this analysis may highlight the negative effect of osmolytes synthesis on plants biomass production due to its high carbon and energy costs. Collectively, these data may suggest that, different patterns of osmolytes accumulation could not explain the biomass response of plants to the IC or DS conditions in our experiment.

3.7 EFFECT OF INTERCROPPING INDEPENDENT FROM WATERING TREATMENT

An improvement in the shoot growth of oilseed rape but reduction of it in barley under IC conditions was observed irrespective the watering treatment in this work (Fig. 1). Response of dry matter production to the neighboring plants has been observed for several intercrop systems. Quite different effects have been found: improvement in both crops (Xue et al., 2016), increase of growth only in one of the crops (Zuo et al., 2003), reduction in both (Inal et al., 2007) or even without biomass response (Zuo et al., 2004). Here, higher biomass production in oilseed rape after 10 weeks intercrop may be partly related to the competition for nutrients with barley favoring growth of oilseed rape. Nevertheless, an improved shoot biomass in oilseed rape upon intercropping with barley has also been observed in the hydroponically grown plants supplied with adequate nutrients provided through repeated replacement of nutrient solution (Sadeghzadeh et al., 2021). This may suggest additional mechanisms for the benefit of oilseed rape from an intercropping system.

Similarly, reduction of biomass in barley under IC conditions cannot only be explained by competition for nutrients. Growth impairment in intercropped plants may be mediated by chemical factors released from the roots of neighboring plants including, but not restricted to, allelochemicals. In an oilseed rape/barley intercrop system, we have observed activation of defense pathways, including phenylpropanoid- and salicylic acid-mediated pathways in barley but not in oilseed rape (Hajiboland, unpublished data). Activation of defense that was also observed in other mixed cropping systems (Schmid et al., 2013; Fu et al., 2015), may divert carbon resources from the growth and is likely the mechanism for reduction of dry matter production in barley under IC conditions. Interspecific relations independent from nutrient acquisition capacity in intercropped systems has attracted much less attention and our knowledge about the underlying mechanisms of belowground interactions is largely limited compared to other types of biotic interactions (Subrahmaniam et al., 2013).

The measured physiological parameters subjected to PCA (Fig. 6) showed a distinct clustering of four treatment combinations in oilseed rape. In barley, in contrast, the physiological parameters relevant to the cropping pattern were not clustered separately under WW conditions. This confirmed again the prominent effect of intercropping in oilseed rape irrespective the watering treatment and suggested that, barley may benefit from IC only under DS conditions.

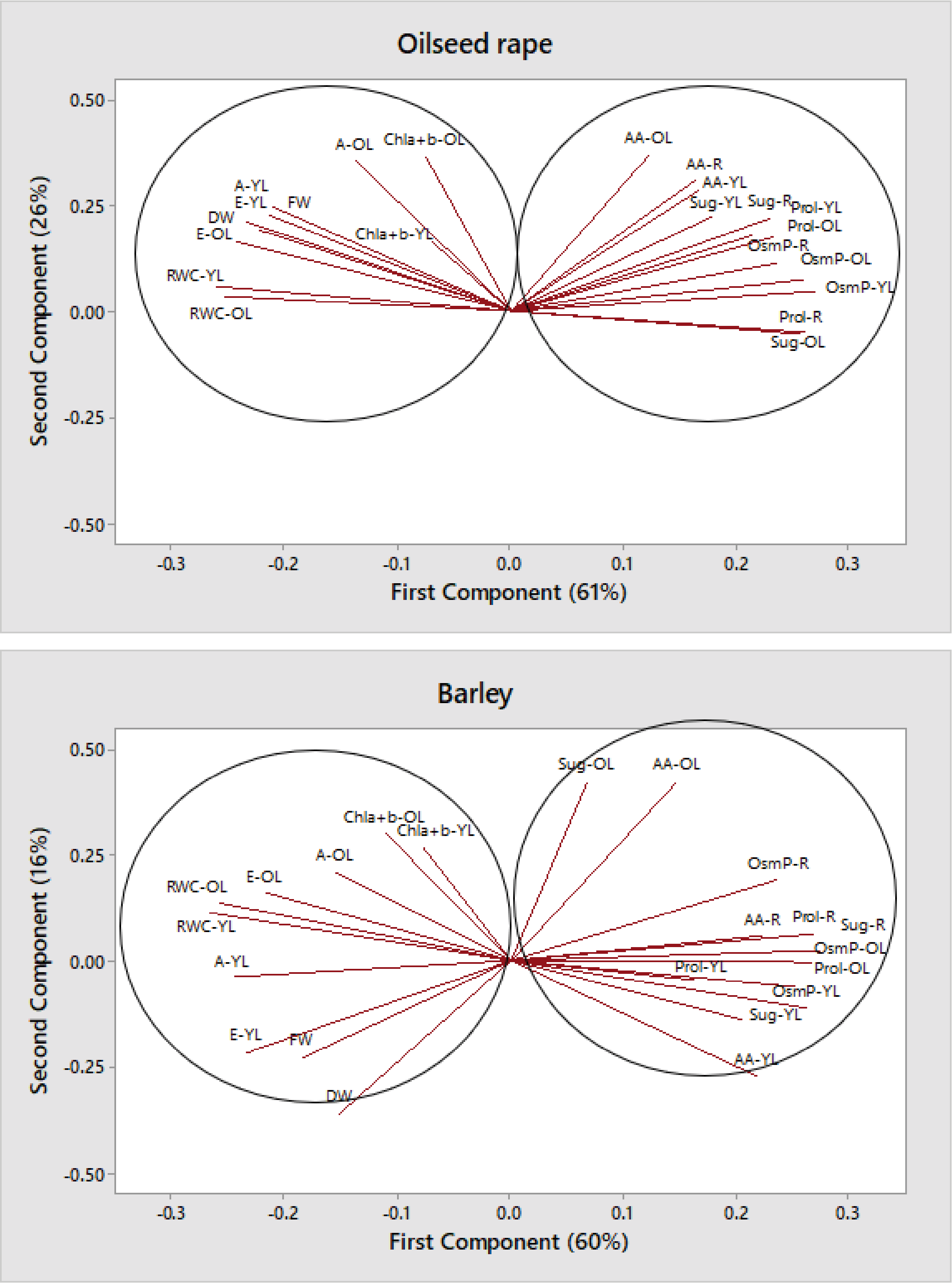

Figure 5: Principal component analysis of various physiological parameters in the young (YL) and old leaves (OL) and roots (R) of oilseed rape and barley cultivated either as monocrop or intercrop under well-watered or drought stress conditions for eight weeks. Abbreviations: Chl (chlorophyll), A: photosynthesis, E: transpiration, FM: fresh mass, DM: dry mass; RWC: relative eater content, AA: concentration of free amino acids, Sug: concentration of soluble sugars, Prol: concentration of proline, Osm: osmotic potential

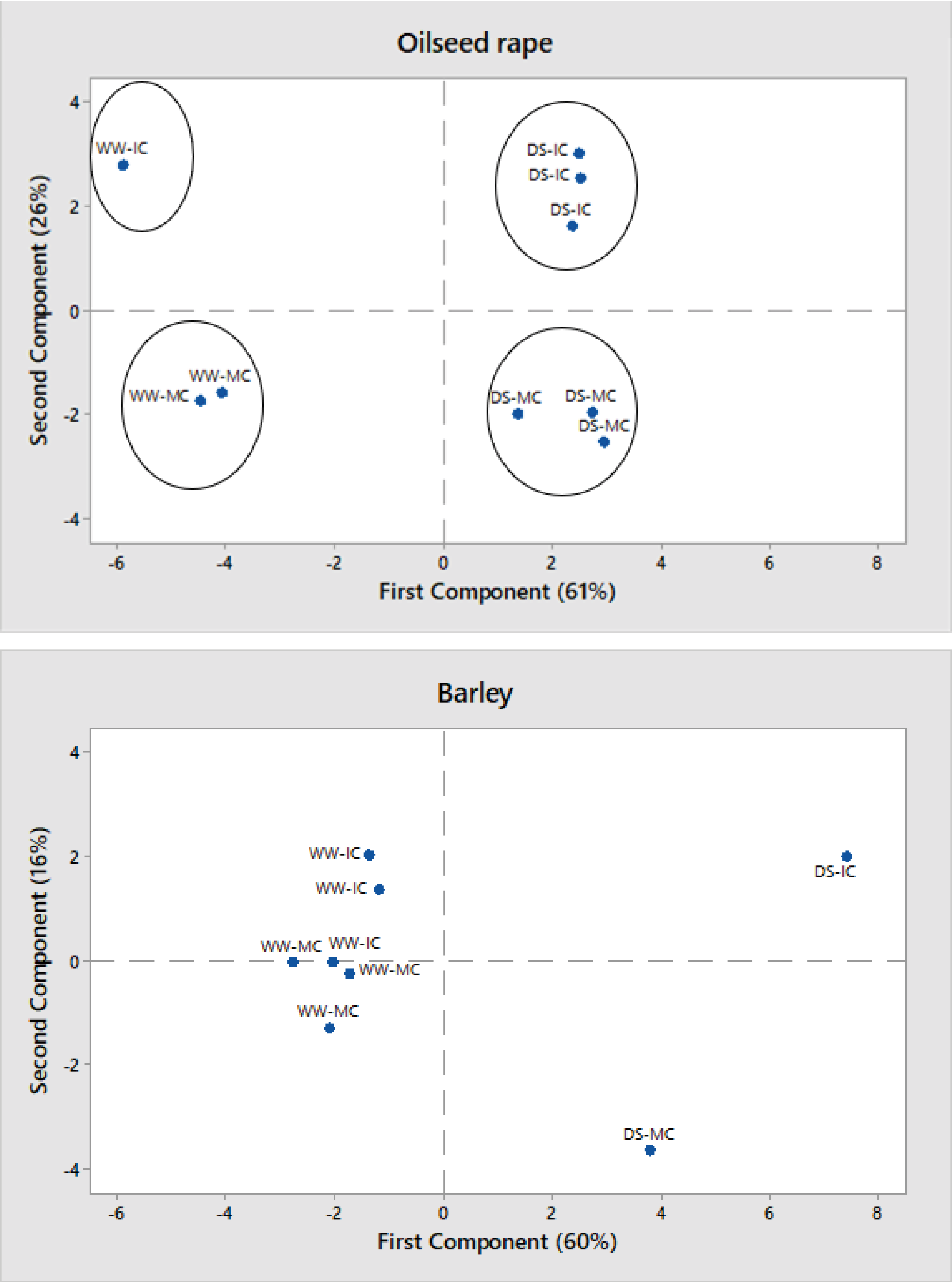

Figure 6: Principal component analysis of various physiological parameters in oilseed rape and barley cultivated either as monocrop (MC) or intercrop (IC) under well-watered (WW) or drought stress (DS) conditions for eight weeks

Fig. 7: Difference in the greenness of the old leaves in oilseed rape cultivated either as monocrop (above) or intercrop (below) with barley under drought stress conditions for eight weeks

3.8 DIFFERENCE BETWEEN THE YOUNG AND OLD LEAVES

The separate analysis of young and old leaves in this work showed differences between these leaves in the two species. The IC-mediated increase in the RWC was observed only in the young leaves of both species. There are evidences on the different response of leaves to drought stress depending on the leaf ontogenetic stage (Chastain et al., 2016). It has been stated that the leaves which develop after imposition of drought stress are more tolerant to water deficit than the old leaves; both in primary photochemistry and carbon reactions (Hajiboland et al., 2014; Chastain et al., 2016). In oilseed rape, further differences in the response to DS and IC between the young and old leaves were observed. The accumulation of proline under DS conditions was much higher in the young than in the old leaves (Table 2).

3.9 LEAF SENESCENCE AS AFFECTED BY INTERCROPPING

The old leaves of intercropped oilseed rape plants retained much better their green colour than the MC plants. This was particularly found under DS conditions (Fig. 7) and was also obvious from the Chl a + b data associated with a higher photosynthesis rate (Table 1). Drought-induced leaf senescence that is characterized by reduction of Chl and photosynthesis rate is an intricate process resulting in remobilization of nutrients to younger leaves thereby contributing to plant fitness (Jan et al., 2019). A direct role in the regulation of drought-induced leaf senescence has been demonstrated for cytokinins and ABA operating at opposite manner (Munné-Bosch and Alegre, 2004). Cytokinin levels that show a positive correlation with the photosynthetic rate and Chl content decrease under drought stress (Munné-Bosch and Alegre, 2004). The mechanism for the IC-mediated prevention of leaf senescence in oilseed rape plants was not addressed here, but could likely be related to an elevated level of cytokinin as was also observed in other belowground root interactions (Chen et al., 2018). Similar to our work on the improvement of Chl and photosynthesis in the oilseed rape, in the peanut/maize intercrop system, a proteomics study showed a three-fold increase in the expression of Rubisco small and large subunits, Rubisco activase and Chl a/b binding proteins compared to monocrop peanut young leaves (Xiong et al., 2013). Our data on the postponing of senescence in the old leaves of oilseed rape by intercropping will putatively increase the leaf area duration in this species and may contribute significantly to the higher biomass production under IC conditions in this species.

4 CONCLUSIONS

Cropping pattern considerably influenced the plants water and osmotic homeostasis under drought stress conditions. Elevated RWC, WUE and an improved osmotic adjustment in both species showed a conspicuous effect of belowground root interaction on plants response to water deficit conditions. Further benefits of IC were higher biomass production and leaf area duration in oilseed rape plants and higher root : shoot ratio in barley. Such plasticity in plant morphological and physiological traits is expected to increase plant performance, canopy photosynthesis and productivity and enhance water capture under intercropping conditions in the field. These results suggest intercropping as a suitable agricultural practice for oilseed rape and barley cultivated under water scarce or rainfed conditions. It is necessary, however, to examine the efficiency of different intercropping patterns for obtaining higher biomass and WUE under field conditions.

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