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SALINITY-INDUCED MODULATION OF PLANT GROWTH AND PHOTOSYNTHETIC PARAMETERS IN FABA BEAN (*VICIA FABA*) CULTIVARS

MUHAMMAD HUSSEIN[1], ALACHEW EMBIALE[1], AZAMAL HUSEN[1], IBRAHIM M. AREF[2] AND MUHAMMAD IQBAL[3*]

[1] *Department of Biology, College of Natural and Computational Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia*
[2]*Department of Plant Production, College of Food & Agricultural Sciences, PO Box 2460, King Saud University, Riyadh 11451, Saudi Arabia*
[3]*Department of Botany, Faculty of Science, Hamdard University, Hamdard Nagar, New Delhi-110062, India*
[*]*Corresponding author's email: iqbalg5@yahoo.co.in; Ph: +91-11-26059688-5530*

Abstract

Salinity is one of the most severe environmental factors limiting the productivity of agricultural crops. The present study assesses salt-tolerant cultivars of *Vicia faba* L.on the basis of their growth, biomass and foliar characteristics. Four levels of salt stress (0, 50, 100 and 150mM) were applied to three selected cultivars. Degaga, Dosha and Hachalu. Results revealed significant differences among the cultivars, salt-stress treatments, and their interaction, indicating the cultivars' variability and differential response to salt stress. Salinity stress adversely affected plant growth, plant water status and biomass production. Salt treatments decreased the chlorophyll *a* and chlorophyll *b* contents, but cultivar Dosha, which was ahead of others in height, leaf number, relative water content, total biomass and leaf-dry-mass ratio, was least affected. Functional leaf characters, such as photochemical efficiency of PSII (maximum quantum yield = *Fv/Fm*), stomatal conductance (*gs*), net photosynthetic rate (*Pn*) and transpiration rate (*E*) were also reduced under salt-stress, and againDosha cultivar did better than others except in *gs.* The relatively less decline in growth, water status, biomass, photosynthetic pigments and functional leaf characters of Dosha exhibits a reasonable tolerance ability of this cultivar, while the other two varieties viz., Degaga and Hachalu proved to be sensitive to salt stress.

Key words: Biomass accumulation; Chlorophyll content; Chlorophyll fluorescence; Faba bean; Photosynthetic rate; Relative water content.

Introduction

Salinity affects about 34 million hectares of land (11% of irrigated area) in the world; an additional 60-80 million hectares are affected by water logging and consequent salinity (Anon., 2012). The salt-affected soils are common in the Rift Valley, Awash Valley and lowland areas of Ethiopia (Gebreselssie, 1993). Nearly 57% of the 4000 ha irrigated land of Melka Sadi Farm (Taddese & Bekele, 1996), entire Melka Werer Research Farm (Haider *et al.*, 1988), and 30% of the Abaya State Farm (Tsige *et al.*, 2000) are salt-affected. Salinity problem is likely to be more severe in the coming years for absence of suitable management practices and a growing tendency of large-scale-irrigation agriculture (Mamo *et al.*, 1996).

Soil-salinity stress is one of the most serious abiotic threats to distribution, survival and productivity of plants. It can disturb a number of biochemical and physiological processes and limit biomass accumulation, which determines the net primary production and growth rate (Tackenberg, 2007; Arshi *et al.*, 2006; 2012; Qureshi *et al.*, 2013; Qiong *et al.*, 2016; Ali & Rab, 2017). Salinity reduces water-absorption ability of plants, and induces metabolic changes similar to those caused by water stress (Hasegawa *et al.*, 2000). The adverse effects on plant growth may be due to ion cytotoxicity and osmotic stress, which cause nutritional deficiencies and metabolic imbalance (Zhu *et al.*, 2002; Arshi *et al.*, 2010a, b; Qureshi *et al.*, 2013).

The osmotic adjustment in leaves contributes to the maintenance of water uptake and cell turgor, allowing for physiological processes like stomatal conductance, photosynthesis and cell expansion (Serraj & Sinclair, 2002; Aref *et al*., 2013). The effect of salinity on photosynthesis may depend on changes in photosynthesizing tissue, disturbance in plant water balance and homoeostatis of Na^+ and Cl^- ions (Munns & Tester, 2008). Photosynthetic inhibition due to salinity is often associated with damage to photochemical efficiency of PS II (Abdeshahian *et al.*, 2010). Different cultivars may show difference in their inherent potential to tolerate salinity stress in terms of their PS II response (Baker, 2008). Chlorophyll fluorescence (*Fv/Fm*)is also a promising indicator of functionality of photosynthetic apparatus in plants (Gomathi & Rakkiyapan, 2011).

Faba bean (*Vicia faba* L.) is a known food crop and an emerging herbal medicine. Being a good source of levadopa (L-dopa), a precursor of dopamine, it has a potential utility the treatment of Parkinson's disease. L-dopa is also a natriuretic agent that can help in managing hypertension; it is also linked to the human libido (Singh *et al.*, 2013). The plant also produces several vitamins and folate, which may protect newborn babies from neural-tube defects, if consumed by the pregnant mothers. Ethiopia is one of its major producers, standing second to China only (Hawitin & Hebblethwaite,1993). It is one of the nine major agro-geographical producing regions of faba bean along with other crops (Tilaye *et al.*,1994; Husen *et al.*, 2012). The wide range of variation that exists among different crop plants and their cultivars may be utilized gainfully for identifying and developing the salt-tolerant candidates. Given this, three faba been (*Vicia faba* L.*)* cultivars were selected for study of their response to salt stress, assuming that they could show differential capacity of tolerance to salinity.

Material and Methods

Experimental site: The experiments were conducted in the University of Gondar, located at 12° 35' 14.19" N, 37° 26' 29.53"E at 2143 m above mean sea level. The annual average of the maximum and the minimum temperature at Gondar, Ethiopia lies around 27°C and 16°C respectively. March to May are the hottest months, with an average maximum temperature of 29°C. Average precipitation in Gondar is about 1161mm per annum, which means a monthly precipitation of 96.75mm. The annual average of relative humidity (RH) is about 56%, the lowest occurring in January-February and the highest in July.

Plant material and soil media: Seeds of three cultivars (Degaga, Dosha and Hachalu) of faba bean, were obtained from Gondar Agricultural Research Centre, and surface sterilized with 80% ethyl alcohol for 15 min, followed by repeated washings with distilled water. The clean seeds of each cultivar were then sown in separate plastic trays containing 75% soil and 25% farmyard manure (FYM) and being watered regularly. After 2 weeks of germination, uniform seedlings chosen from each cultivar were transferred separately to plastic pots (8 cm width x 16 cm height) filled with 1.5kg soil and 500g FYM in 3:1 ratio, sown at a depth of 2 cm and irrigated at 100% field capacity (FC) daily with tap water for the next 2 weeks supposedly a period of plant acclimatization. The soil in pots was sandy loam (62.56% sand, 14.88% clay and 22.56% silt), with pH 7.23, and EC 0.69 ms/cm.

Experimental design and salt treatments: After acclimatization (15 days), pots were placed in a completely randomised design (CRD) and salt treatments were applied in 5 replicates. The pots were exposed to four concentrations (0, 50, 100 and 150mM) of NaCl; those with 0 concentration of salt (untreated soil + FYM only) were used as control. Salt solutions were supplied to each cultivar along with tap water (300 ml per pot every second day up to 4 weeks). Sampling was done 30 days after treatment, when plants were about 45-day old.

Plant growth and leaf characteristics: After 4 week application of salt-stress, growth parameters and leaf characteristics were measured in the control (0mM) and salt-treated (50, 100 and 150mM) plants of Degaga, Dosha and Hachalu cultivars of faba bean. Theseedlingswere gently uprooted for recording the plant length (cm), number of branches, and the size, area and number of opened leaves. Ground-line basal diameter (mm) of stem was measured with an electronic digital caliper. The width (mm), length (mm) and area (mm^2) of leaves were measured with the help of leaf area meter (AM 300, ADC Bio Scientific Limited, U.K.). Five replications were used for determining each parameter.

Biometric study: After 4-week exposure to salt-stress, plants of each cultivar from each treatment were harvested and divided in roots, stems and leaves. For each sample, five replications were used. Roots were washed carefully with tap water, and excess water was removed using blotting paper. All the plant parts were oven-dried separately at 85ºC for 2 days when the weight became constant. Root biomass (RB), stem biomass (SB) and leaf biomass (LB) were then determined by using electronic digital balance (Citizen Scale, CY510, Poland). From these data, the following biometric traits were calculated:

- Total biomass (TB) = RB+ LB+SB
- Root to shoot ratio (R/S) = RB/ (SB+LB)
- Root dry mass ratio (RMR) = RB/TB
- Stem dry mass ratio (SMR) = SB/TB
- Leaf dry mass ratio (LMR) = LB/TB

Chlorophyll analysis: Chlorophyll content was analyzed in randomly collected leaves of each cultivar, using three replications per cultivar. Approximately, 100 mg of fresh leaves was used, and chlorophyll pigments were extracted with 80% acetone. The absorbance was measured at 645 and 663nm, using a T60 UV/VIS spectrophotometer (PG Instruments Limited, England). Thereafter, chlorophyll *a* and chlorophyll *b* were calculated according to Arnon (1949) and expressed in µg ml^{-1}.

Relative water content: Water status of leaf was determined in fully developed leaves of the control and salt-stressed plants by measuring the relative water content (RWC). Three replications per cultivars were used. Leaf samples were weighed immediately after harvesting, to obtain fresh weight and then kept overnight in distilled water at 5°C in the dark, before obtaining their turgid weight (TW). The material was then oven-dried at 85°C for 48h,and dry weight (DW) obtained. The relative water content was calculated as:

$$RWC = \{(FW - DW) \div (TW - DW)\} \times 100$$

Physiological measurements: Leaf gas exchange was analyzed between 9.00-11.00 AM to determine the stomatal conductance (*gs*), net photosynthetic rate (*Pn*) and transpiration rate (*E*), using a portable leaf gas exchange system (ADC Bio Scientific Limited, U.K.) on fully expanded attached leaves. The equipment was used with the following specifications/adjustments: leaf surface area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 371µmol mol^{-1}, temperature of leaf chamber (Tch) 25 to 28°C, molar air flow per m^2 of leaf surface (Us) 296mol m^{-2} s^{-1}, leaf chamber volume gas flow rate (v) 400ml m^{-1}, ambient pressure (P) 97.95kPa, PAR (Q_{leaf}) at leaf surface up to 770µmol m^{-2} s^{-1}. Chlorophyll fluorescence was also analysed for leaves from each treatment, using a portable Multi-Mode Chlorophyll Fluorometer (0S5p Opti-Sciences, Inc., USA). Forfluorescence measurement, the upper surface of leaf was pre-darkened with leaf clips for 30 minutes to ensure complete relaxation of all reaction centres. The basal non-variable chlorophyll fluorescence (*Fo*), the maximal fluorescence induction (*Fm*) and the variable fluorescence (*Fv*) were determined, and the photochemical efficiency of PSII (the maximum quantum yield = *Fv/Fm*) was estimated by the ratio *Fv/Fm* = (*Fm* – *Fo*)/ *Fm* (Genty *et al.,* 1989).

Statistical analysis: Statistical analysis of data was performed with version 16.0 Statistical Package for Social Sciences (SPSS) software package (SPSS Inc., Illinois, USA). The data were subjected to two-way (Cultivars:

Degaga, Dosha and Hachalu x Salt-stress treatments: 0, 50, 100 and 150mM) analysis of variance (ANOVA) to determine the significant difference among treatments and cultivars. Differences among the mean values were assessed by Least Significant Differences (LSD) at significance level $p<0.05$.

Results

Plant growth: Analysis of variance has revealed that salt treatments significantly affected all growth and leaf characteristics. Effect of cultivars was significant only for height, number of leaf, leaf area and leaf width. However, the cultivars x salt-treatments interaction effect was significant for all the parameters studied (Table 1). Plant height, number of leaves, leaf area and leaf width attained the maximum in cultivar Dosha (Table 2). As the salinity level increased, growth parameters declined significantly (Table 2). The control plants were taller in comparison to NaCl-treated plants in each cultivar. In comparison to control, plant height was reduced by 44%, 40% and 49% (Fig. 1a), whereas the basal diameter increment declined by 51%, 27% and 34% (Fig. 1b) in cultivars Degaga, Dosha and Hachalu, respectively, at the highest (150mM) level of salt stress. Leaf production was also low under salinity treatments, being the lowest at the highest (150mM) salinity level; percent variation from the control being 22%, 29% and 38% at this level for the respective three cultivars (Fig. 1c). Number of branches decreased by 49%, 40% and 55% at the highest stress level in Degaga, Dosha and Hachalu, respectively (Fig. 1d).Leaf area expansion was reduced, with reference to the control, by 16%, 11%, and 28% (Fig. 1e), leaf width by 4%, 10%, and 12% (Fig. 1f), while the leaf length by 30%, 25% and 26% in cv. Degaga, Dosha and Hachalu, respectively (Fig. 1g). Cultivar Dosha was less affected by salinity in the growth parameters studied, except for the number and the width of the leaf (Table 2, Fig. 1a-g).

Plant biometry: Plant biometry in terms of biomass production was significantly affected by salt stress, as shown by the analysis of variation (Table 1). Effect of cultivars was significant for total biomass (TB), stem dry mass ratio (SMR) and leaf dry mass ratio (LMR). However, interactive effect of salt treatments x cultivars was significant for all the parameters studied (Table 1). Cultivar Dohsa exhibited significantly higher TB, SMR and LMR in comparison to the other cultivars. TB, root-to-shoot ratio (R/S), root dry mass ratio (RMR), SMR and LMR was reduced under salt stress in a dose-dependent manner. Control plants exhibited the higher TB, R/S, RMR, SMR and LMRin comparison to the NaCl-treated plants (Table 3). Of the various NaCl concentrations, 150mM was most effective in terms of reduction of plant biometry for each cultivar. In comparison to control, the reduction in TB at 150mM was up to 77%, 57% and 58% in Degaga, Dosha and Hachalu, respectively (Fig. 2a). Similarly, R/S was reduced by 59%, 45% and 48%, RMR by 54%, 44% and 60%, SMR by 26%, 38% and 34%, and LMR by 22%, 13% and 18% in cv. Degaga, Dosha and Hachalu, respectively, as compared with the control (Fig. 2b-e). Except for SMR, Dosha cultivar could maintain less percent variation and registered the highest mean values for various parameters at all the NaCl concentrations tested (Table 3, Fig. 2a-e).

Table 1. ANOVA results on the effect of cultivars, salt treatments and their combination for growth, biometric traits, chlorophyll contents and physiological parameters.

Parameters	Cultivars			Salt treatments			Cultivars x Salt treatments		
	MSS	p<0.05	p<0.01	MSS	p<0.05	p<0.01	MSS	p<0.05	p<0.01
Height (cm)	49.23	*	-	877.90	-	**	34.13	*	-
Stem basal diameter (mm)	2.19	-	-	24.89	*	-	1.06	*	-
Number of leaf	106.20	-	**	210.22	-	**	89.59	-	**
Number of branch	41.28	-	-	220.99	*	-	5.46	*	-
Leaf area (mm^2)	4231.67	-	**	85921.72	-	**	3714.28	-	**
Leaf width (mm)	4.12	*	-	34.22	*	-	2.73	*	-
Leaf length (mm)	254.07	-	-	629.37	*	-	116.35	*	-
Total biomass	2.10	-	**	8.67	-	**	0.87	-	**
Root/Shoot ratio	0.025	-	-	0.064	*	-	0.002	*	-
Root dry mass ratio	0.011	-	-	0.012	*		0.022	*	-
Stem dry mass ratio	0.058	*	-	0.008	-	**	0.036	*	-
Leaf dry mass ratio	0.019	-	**	0.10	-	**	0.10	**	-
Chlorophyll *a*	6.19	*	-	1.65	-	**	0.17	-	**
Chlorophyll *b*	6.19	*	-	1.65	*	-	0.17	*	-
Relative water content (%)	664.01	*	-	128.79	-	**	36.09	-	**
Chlorophyll fluorescence	0.003	*	-	0.020	*	-	0.003	*	-
Photosynthetic rate (µmol $m^{-2}s^{-1}$)	30.51	*	-	42.76	-	**	7.03	*	-
Stomata conductance (mol $m^{-2}s^{-1}$)	0.001	-	-	0.009	*	-	0.001	*	-
Transpiration rate (m mol $m^{-2}s^{-1}$)	0.004	*	-	4.064	*	-	0.493	*	-

MSS mean square value, * and ** significance level at $p<0.05$ and $p<0.01$, respectively

Table 2. Effects of salt-stress treatments on different growth and leaf characteristic features in selected cultivars of *Vicia faba*.

Parameters	Cultivars	Salt-stress treatments			
		0mM	50mM	100mM	150mM
Height (cm)	Degaga	30.00 ± 1.84^{b}	24.89 ± 1.25^{c}	22.89 ± 1.44^{c}	16.84 ± 1.54^{d}
	Dosha	32.00 ± 1.32^{a}	27.89 ± 1.33^{b}	25.22 ± 1.37^{c}	19.11 ± 1.47^{d}
	Hachalu	30.56 ± 1.62^{b}	26.44 ± 1.64^{c}	24.78 ± 1.21^{c}	15.67 ± 1.83^{d}
Stem basal diameter (mm)	Degaga	5.54 ± 0.25^{a}	4.52 ± 0.28^{b}	3.47 ± 0.21^{c}	2.72 ± 0.25^{d}
	Dosha	5.45 ± 0.24^{a}	4.87 ± 0.22^{b}	4.10 ± 0.23^{c}	3.95 ± 0.21^{d}
	Hachalu	4.52 ± 0.23^{a}	3.93 ± 0.23^{b}	3.40 ± 0.27^{c}	2.97 ± 0.24^{d}
Number of leaf	Degaga	36.78 ± 3.04^{b}	29.44 ± 3.28^{c}	29.00 ± 2.94^{c}	28.67 ± 2.93^{c}
	Dosha	54.70 ± 2.67^{a}	45.30 ± 3.01^{b}	41.56 ± 2.73^{b}	38.75 ± 2.67^{c}
	Hachalu	41.22 ± 3.11^{b}	32.22 ± 2.89^{c}	27.67 ± 3.14^{c}	25.33 ± 2.56^{d}
Number of branch	Degaga	13.11 ± 3.98^{a}	10.67 ± 3.43^{b}	10.11 ± 2.82^{b}	6.67 ± 1.73^{c}
	Dosha	15.30 ± 3.42^{a}	13.22 ± 2.19^{a}	11.67 ± 2.87^{b}	9.11 ± 1.89^{c}
	Hachalu	14.33 ± 3.35^{a}	12.11 ± 3.77^{b}	9.44 ± 2.62^{b}	6.44 ± 2.01^{c}
Leaf area (mm^2)	Degaga	13547 ± 988^{b}	13139 ± 845^{bc}	12632 ± 763^{bc}	11270 ± 472^{c}
	Dosha	15717 ± 756^{a}	14761 ± 561^{ab}	14005 ± 472^{bc}	13991 ± 482^{bc}
	Hachalu	14350 ± 472^{b}	11655 ± 472^{bc}	10464 ± 387^{cd}	10294 ± 782^{cd}
Leaf width (mm)	Degaga	8.42 ± 0.87^{b}	8.33 ± 0.77^{b}	8.08 ± 0.53^{b}	8.02 ± 0.62^{b}
	Dosha	9.04 ± 0.81^{a}	8.81 ± 0.63^{b}	8.17 ± 0.79^{b}	8. 10 ± 0.58^{b}
	Hachalu	8.13 ± 0.69^{b}	8.11 ± 0.62^{b}	8.04 ± 0.41^{b}	7.08 ± 0.72^{c}
Leaf length (mm)	Degaga	178.57 ± 12.84^{a}	162.57 ± 10.23^{ac}	160.43 ± 12.20^{bc}	125.60 ± 10.62^{bc}
	Dosha	200.40 ± 10.28^{a}	197.83 ± 9.56^{ac}	191.40 ± 11.07^{bc}	149.67 ± 9.37^{bc}
	Hachalu	184.77 ± 8.72^{a}	183.90 ± 8.34^{ac}	150.00 ± 9.98^{bc}	136.13 ± 8.94^{bc}

Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

Table 3. Effects of salt-stress treatments on the biometry in selected cultivars of *Vicia faba*.

Parameters	Cultivars	Salinity- stress treatments			
		0mM	50mM	100mM	150mM
Total biomass	Degaga	2.38 ± 0.18^{b}	1.03 ± 0.22^{c}	0.78 ± 0.20^{d}	0.54 ± 0.22^{d}
	Dosha	2.42 ± 0.12^{a}	1.58 ± 0.19^{b}	1.08 ± 0.14^{c}	1.02 ± 0.21^{c}
	Hachalu	1.22 ± 0.15^{c}	1.03 ± 0.20^{c}	0.84 ± 0.18^{d}	0.51 ± 0.18^{d}
Root/Shoot ratio	Degaga	0.32 ± 0 .04^{a}	0.18 ± 0.05^{b}	0.22 ± 0.04^{b}	0.13 ± 0.05^{c}
	Dosha	0.33 ± 0.05^{a}	0.24 ± 0.02^{a}	0.22 ± 0.03^{b}	0.18 ± 0.03^{b}
	Hachalu	0.25 ± 0.03^{a}	0.19 ± 0.04^{b}	0.18 ± 0.06^{b}	0.13 ± 0.04^{c}
Root dry mass ratio	Degaga	0.24 ± 0.03^{a}	0.15 ± 0.04^{b}	0.12 ± 0.03^{b}	0.11 ± 0.04^{c}
	Dosha	0.25 ± 0.08^{a}	0.21 ± 0.03^{a}	0.17 ± 0.02^{b}	0.14 ± 0.03^{b}
	Hachalu	0.25 ± 0.04^{a}	0.12 ± 0.01^{b}	0.15 ± 0.06^{b}	0.10 ± 0.01^{c}
Stem dry mass ratio	Degaga	0.45 ± 0.01^{b}	0.39 ± 0.05^{c}	0.38 ± 0.04^{c}	0.33 ± 0.05^{d}
	Dosha	0.58 ± 0.04^{a}	0.43 ± 0.03^{b}	0.39 ± 0.06^{c}	0.36 ± 0.02^{c}
	Hachalu	0.44 ± 0.02^{b}	0.37 ± 0.01^{c}	0.36 ± 0.03^{c}	0.29 ± 0.04^{d}
Leaf dry mass ratio	Degaga	0.46 ± 0.05^{b}	0.44 ± 0.04^{b}	0.43 ± 0.07^{c}	0.36 ± 0.06^{d}
	Dosha	0.51 ± 0.06^{a}	0.49 ± 0.02^{a}	0.45 ± 0.03^{b}	0.44 ± 0.04^{b}
	Hachalu	0.44 ± 0.07^{b}	0.43 ± 0.06^{c}	0.42 ± 0.02^{c}	0.36 ± 0.07^{d}

Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

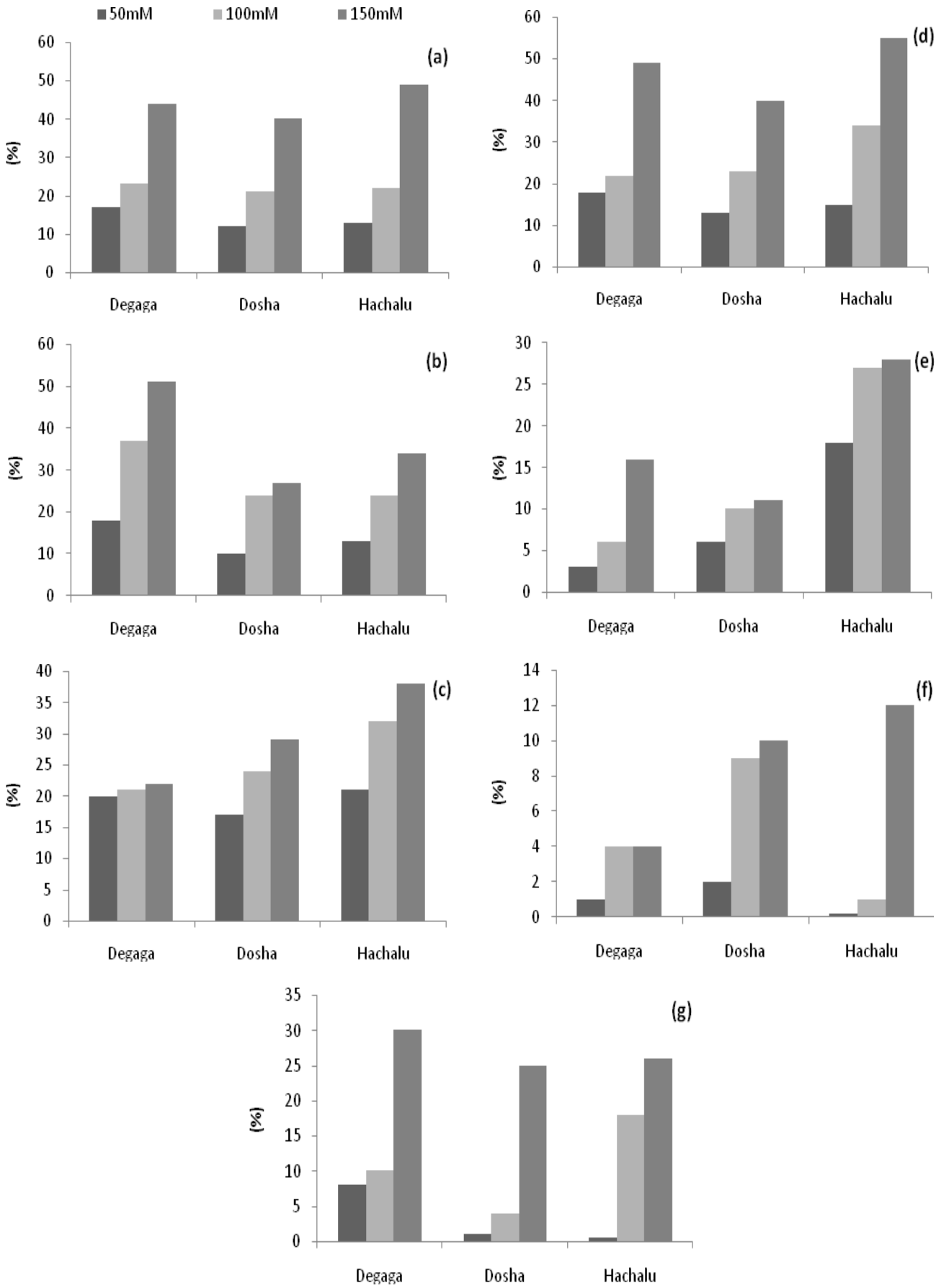

Fig. 1. Percent variation from the control (0mM NaCl), as observed under high salinity (150mM NaCl) for (a) plant height (b) stem basal diameter (c) number of leaves (d) number of branches (e) leaf area (f) leaf width and (g) leaf length in Degaga, Dosha and Hachalu cultivars of *Vicia faba*.

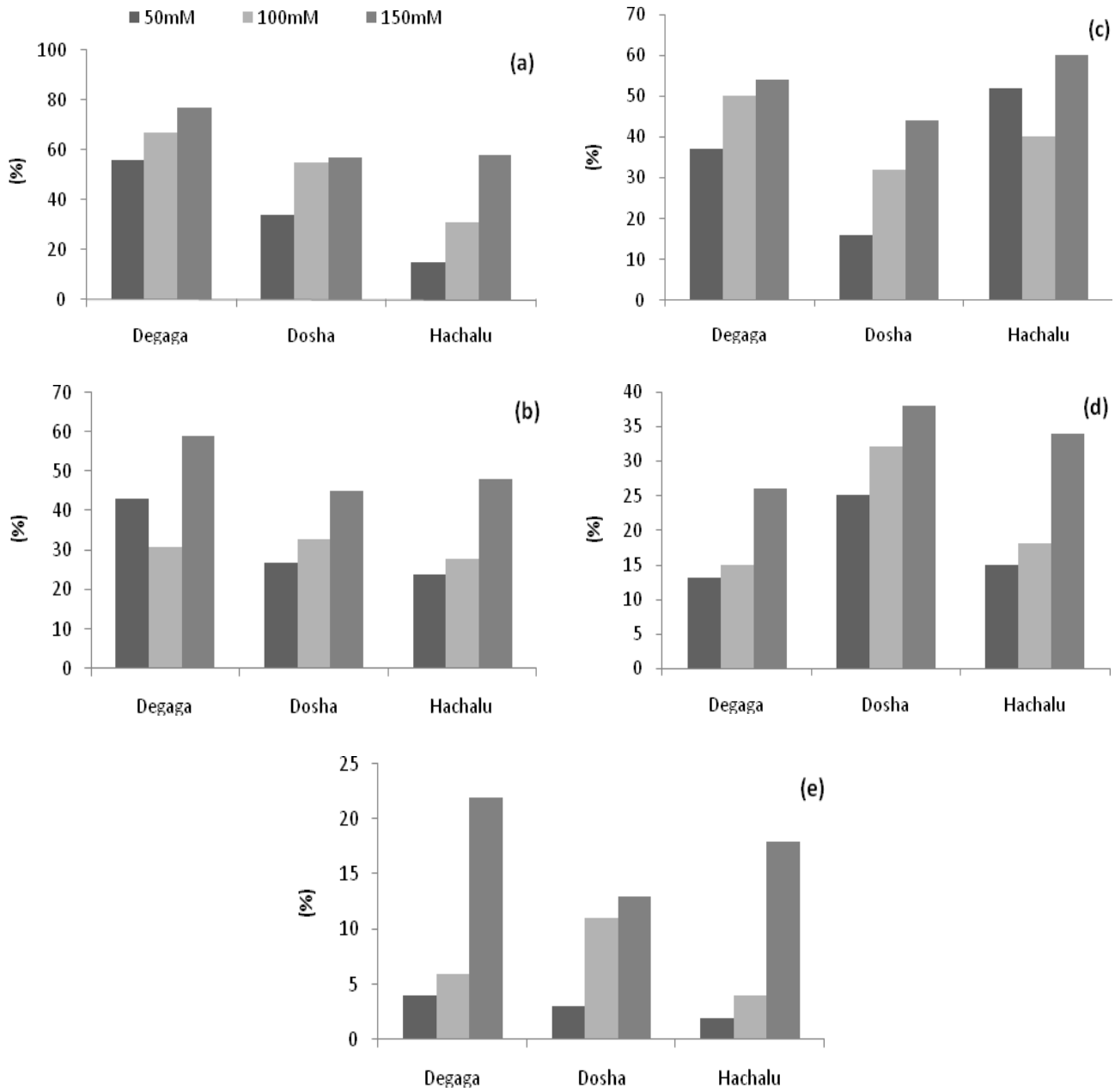

Fig. 2. Percent variation from the control (0mM NaCl), as observed under high salinity (150mM NaCl) for (a) total biomass (b) root/shoot ratio (c) root dry mass ratio (d) stem dry mass ratio and (e) leaf dry mass ratio in Degaga, Dosha and Hachalu cultivars of *Vicia faba*.

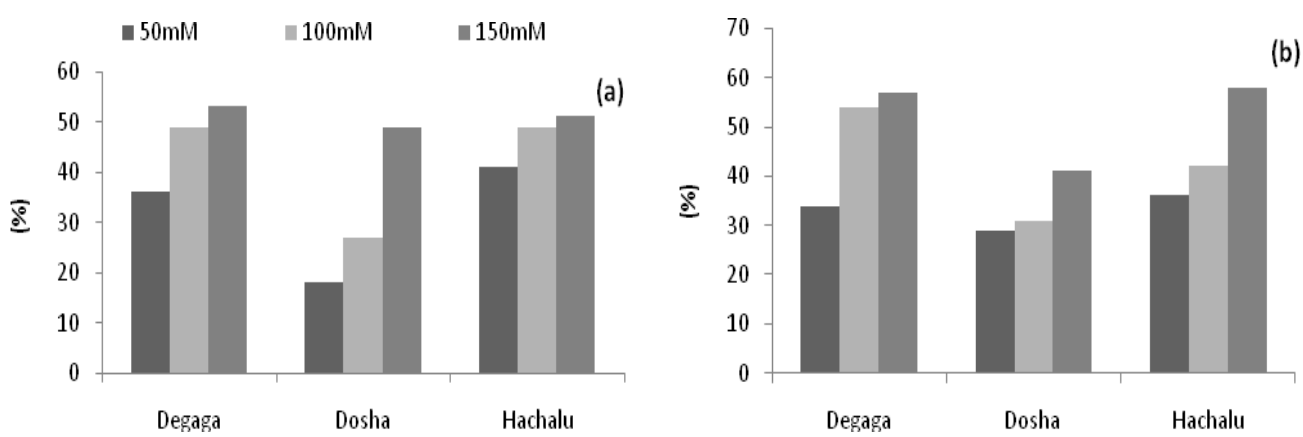

Fig. 3. Percent variation from the control (0mM NaCl), as observed under high salinity (150mM NaCl) for (a) chlorophyll *a* and (b) chlorophyll *b* in Degaga, Dosha and Hachalu cultivars of *Vicia faba*.

Table 4. Effects of salt-stress treatments on the photosynthetic pigments in selected cultivars of *Vicia faba*.

Photosynthetic pigments (μg ml^{-1})	Cultivars	Salinity-stress treatments			
		0mM	50mM	100mM	150mM
Chlorophyll *a*	Degaga	3.37 ± 0.21 ^{b}	2.13 ± 0.161^{c}	1.70 ± 0.14^{c}	1.57 ± 0.14^{d}
	Dosha	3.90 ± 0.13 ^{a}	3.17 ± 0.11 ^{b}	2.44 ± 0.11 ^{c}	1.98 ± 0.16 ^{c}
	Hachalu	3.35 ± 0.15^{b}	1.97 ± 0.18 ^{c}	1.70 ± 0.17 ^{c}	1.63 ± 0.12 ^{d}
Chlorophyll *b*	Degaga	5.83 ± 0.30 ^{b}	3.80 ± 0.14 ^{c}	2.66 ± 0.13 ^{d}	2.50 ± 0.20 ^{d}
	Dosha	6.23 ± 0.23 ^{a}	4.38 ± 0.27 ^{b}	4.28 ± 0.16 ^{b}	3.66 ± 0.14 ^{c}
	Hachalu	5.60 ± 0.26 ^{b}	3.58 ± 0.23 ^{c}	3.22 ± 0.27 ^{d}	2.32 ± 0.16 ^{d}

Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

Table 5. Effects of salt-stress treatments on the various physiological activities in selected cultivars of *Vicia faba*.

Physiological parameters	Cultivars	Salinity- stress treatments			
		0mM	50mM	100mM	150mM
Relative water content (%)	Degaga	67.00 ± 4.25 ^{b}	56.10 ± 4.51 ^{c}	55.10 ± 3.29 ^{c}	50.33 ± 4.14 ^{d}
	Dosha	70.97 ± 3.43 ^{a}	59.50 ± 3.78 ^{b}	58.27 ± 3.67 ^{c}	54.17 ± 4.03 ^{c}
	Hachalu	66.67 ± 3.67 ^{b}	58.33 ± 3.82 ^{c}	51.00 ± 3.91 ^{c}	39.13 ± 2.94^{d}
Chlorophyll fluorescence	Degaga	0.74 ± 0.02^{b}	0.72 ± 0.01 ^{c}	0.67 ± 0.01 ^{d}	0.59 ± 0.02 ^{e}
	Dosha	0.77 ± 0.01 ^{a}	0.74 ± 0.02 ^{b}	0.72 ± 0.03 ^{c}	0.69 ± 0.01 ^{d}
	Hachalu	0.73 ± 0.01 ^{b}	0.71 ± 0.01 ^{c}	0.68 ± 0.01 ^{d}	0.65 ± 0.01 ^{e}
Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})	Degaga	4.26 ± 0.45 ^{b}	3.23 ± 0.33 ^{c}	2.77 ± 0.45 ^{d}	2.63 ± 0.34 ^{d}
	Dosha	6.35 ± 0.35 ^{a}	4.98 ± 0.48 ^{b}	4.43 ± 0.41 ^{b}	3.97 ± 0.37 ^{c}
	Hachalu	4.62 ± 0.45 ^{b}	3.21 ± 0.41 ^{c}	3.35 ± 0.37 ^{c}	2.70 ± 0.42 ^{d}
Stomata conductance (mol m^{-2}s^{-1})	Degaga	0.08 ± 0.002 ^{a}	0.02 ± 0.001 ^{b}	0.01 ± 0.002 ^{b}	0.01 ± 0.002 ^{b}
	Dosha	0.11 ± 0.003 ^{a}	0.06 ± 0.002 ^{a}	0.03 ± 0.002 ^{b}	0.02 ± 0.001 ^{b}
	Hachalu	0.06 ± 0.001 ^{a}	0.03 ± 0.001 ^{b}	0.02 ± 0.001 ^{b}	0.01 ± 0.001 ^{b}
Transpiration rate (m mol m^{-2}s^{-1})	Degaga	1.43 ± 0.41 ^{b}	0.63 ± 0.07 ^{c}	0.57 ± 0.13 ^{c}	0.18 ± 0.09 ^{d}
	Dosha	2.35 ± 0.32 ^{a}	1.65 ± 0.30 ^{b}	0.58 ± 0.15 ^{c}	0.40 ± 0.11 ^{c}
	Hachalu	1.93 ± 0.42 ^{b}	1.01 ± 0.12 ^{c}	0.70 ± 0.11 ^{c}	0.17 ± 0.05 ^{d}

Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

Photosynthetic pigments: Table 1 shows that salt treatments, cultivars and cultivars x salt-treatments interaction significantly affected chl *a* and chl *b* contents. Compared with the control, the chlorophyll contents were significantly lower in salt-treated plants (Table 4). Cultivar Dohsa possessed the highest chl *a* and chl *b* contents among the three cultivars (Table 4). The contents of both chlorophylls consistently decreased in all the three cultivars with increase in the salt stress. In comparison to control, chl *a* declined by 53%, 49% and 51%, while chl *b* by 57%, 41% and 58% in cv Degaga, Dosha and Hachalu, respectively at 150mM salinity level (Fig. 3a, b). Thus, cv Dosha retained the maximum chlorophyll content among the three cultivars (Table 4, Fig. 3a, b).

Leaf water status: Analysis of variance demonstrates that the salt treatments, cultivars and cultivars x salt- treatments interaction significantly affected the relative water content (RWC) (Table 1). Compared to the control, RWC was reduced in salinity-affected plants; the degree of reduction increasing with the increase in salinity level (Table 5). At 150mM, the reduction was around 24%, 23% and 41% in Degaga, Dosha and Hachalu, respectively (Fig. 4). Thus, cv Dosha had the maximum RWC among the cultivars examined (Table 5). It tried to maintain RWC at the highest NaCl treatment, and gave a statistically similar response at 100 and 150mM NaCl; which was not the case with Degaga and Hachalu (Table 5).

Physiological traits: Analysis of variance indicates that the salt treatments significantly affected the photochemical efficiency of PS II (*Fv/Fm*), stomatal conductance (*gs*), net photosynthetic rate (*Pn*) and transpiration rate (*E*). Effect of cultivars and the cultivars x salt-treatments interaction was also significant for these traits, except for *gs* (Table 1). A significant decrease in *Fv/Fm*, *gs*, *Pn* and *E* was measured, especially with high NaCl level. The *Fv/Fm*value was greater in the control than in treated plants. However, it was statistically insensitive in cv Dosha (Table 5). At 150mM, it varied from the control by 20%, 10% and 11% in Degaga, Dosha and Hachalu, respectively (Fig. 5a). In all cultivars, *Pn* suffered a significant reduction which deepened with increase in salinity level; cv Dosha was superior to others. At 100mM salt concentration, reduction in *Pn* lay around 38%, 37% and 41% at 150mM salt concentration, in Degaga, Dosha and Hachalu, respectively, compared to the control (Fig. 5b). The level of *gs* also declined with increasing degree of salinity; the maximum decline recorded at 150mM salt concentration was 87%, 81% and 83% in the three cultivars respectively (Fig. 5c). *E* was significantly greater in Dosha than in other cultivars. However, it decreased significantly under salt stress. The reduction was 87%, 83%, and 91% at 150mM salt concentration in the three cultivars respectively (Fig. 5d). By all accounts, cultivar Dosha was least affected under salinity, thus showing an edge over Degaga and Hachalu (Table 5, Fig. 5a-d).

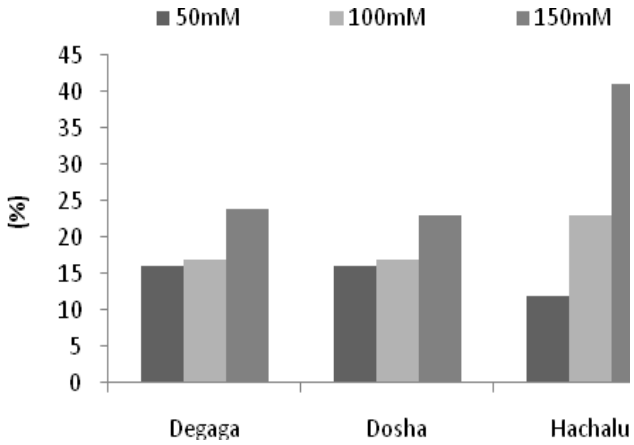

Fig. 4. Percent variation from the control (0mM NaCl), as observed under high salinity (150mM NaCl) for relative water content in Degaga, Dosha and Hachalu cultivars of *Vicia faba*.

Discussion

Plants are exposed to a number of abiotic factors including salinity, which has adverse effects on plant growth and yield parameters (Arshi *et al.*, 2012; Mohsen *et al.*, 2013; Husen *et al*., 2016; Xie *et al.*, 2016; Waheed *et al.*, 2016). In the present study, higher concentration of NaCl caused the maximum inhibitive effect on plant height, number of leaf and leaf area; cv Dosha was less sensitive than others. The shoot and root growth inhibition is a common response to salinity, and plant-growth rate is one of the most important agricultural indices of salt-stress tolerance (Munns *et al.*, 2000; Arshi *et al.*, 2010a; 2012; Hakeem *et al.*, 2012). Deleterious effects of salinity on plant growth stem from limited availability of soil water to roots, low osmotic potential of soil solution, nutritional imbalance, specific ion toxicity, or a combination of these factors. In all stressful conditions, water availability to plant cells is restricted. Therefore, as the first response, cells try to save the available water by avoiding active growth. Saline solution initially creates a water potential imbalance between apoplast and symplast. It leads to a turgor decline, which, if severe enough, causes growth reductions (Sangwan *et al.*, 1994; Bohnert *et al.*, 1995). When turgor decline exceeds the threshold of the cell wall, growth stops. Cellular dehydration begins when water-potential difference goes higher than can be compensated for by the turgor loss. These phenomena finally lead to overall reductions in growth and dry-weight accumulation(Sabir *et al.*, 2012), as observed by us also. The leaf RWC decreased with increase in salinity levels, as observed earlier by Fidalgo *et al.* (2004) and Sekmen & Susumu (2007). However, compared to other cultivars, Dosha had a higher RWC. According to Katerji *et al.* (1997), a decrease in RWC indicates a loss of turgor that results in a limited water availability for cell-extension processes.

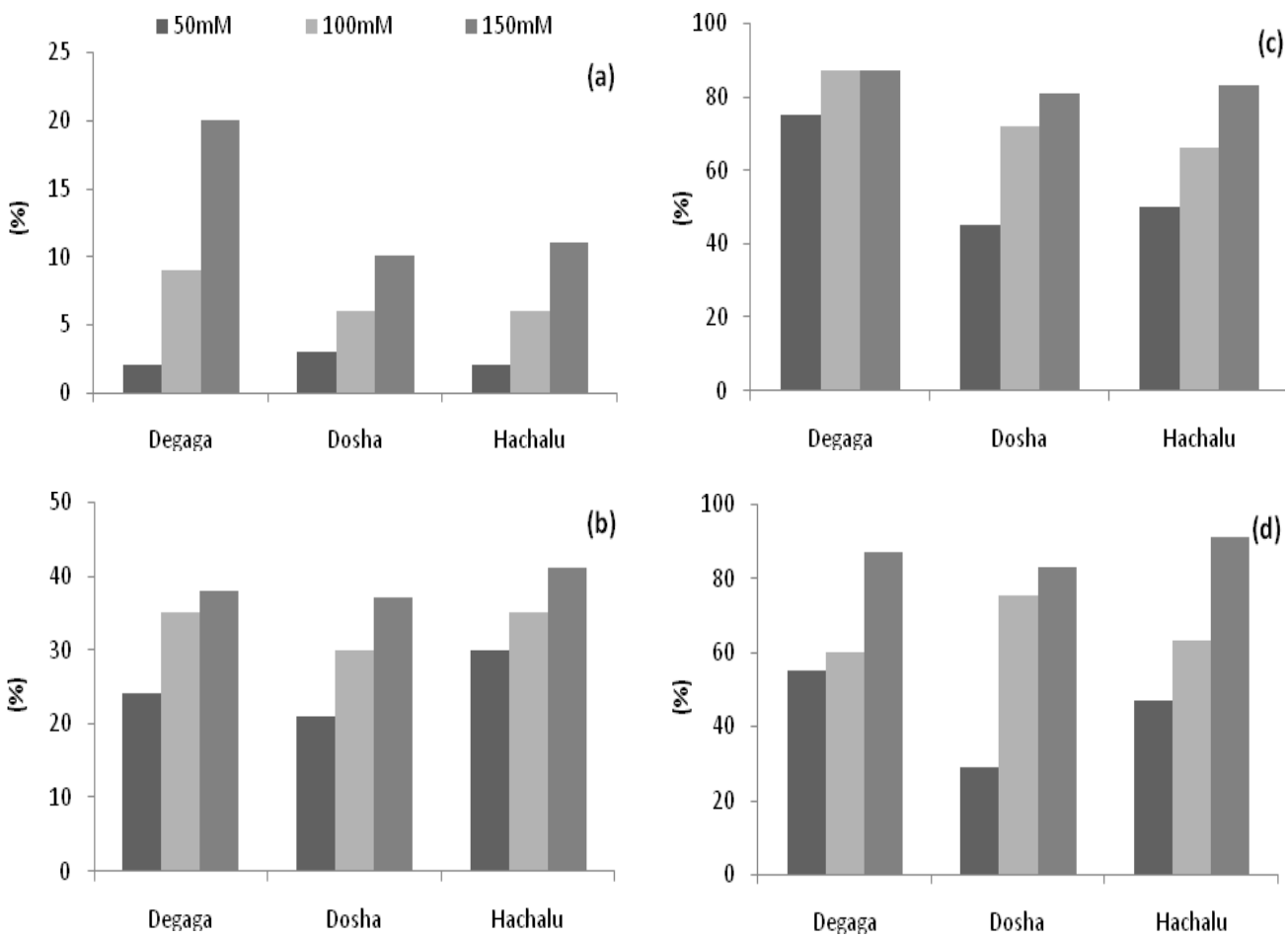

Fig. 5. Percent variation from the control (0mM NaCl), as observed under high salinity (150mM NaCl) for (a) chlorophyll fluorescence (b) net photosynthetic rate (c) stomatal conductance and (d) transpiration rate in Degaga, Dosha and Hachalu cultivars of *Vicia faba*.

Salinity also affects the biomass of different plant organs. Significant reduction in size and number of leaves is linked to a reduction in biomass accumulation. The salinity-caused reduction in total leaf area may be linked to the decline of leaf turgor. Total biomass was greater in cultivar Dosha, while other biometric parameters responded uniformly in all the three cultivars. In general, reduction of biomass is positively correlated with increase in salinity (Soussi *et al.,* 2001; Arshi *et al.,* 2005, 2012), possibly because salinity can affect external water potential and ion toxicity/imbalance (Hasegawa *et al.,* 2000). A high NaCl content of the soil reduces the uptake of mineral nutrients, particularly of K^+, Mg^{2+} and Ca^{2+}. In consequence, biomass production and hence the plant growth rate are retarded (Pessarakli & Huber, 1991; Pessarakli, 1994). Plants respond to environmental stress through osmotic adjustment, normally by augmenting the concentration of Na^+ and Cl^- in tissues, although accumulations of inorganic ions may cause cell damage. Excess of Na^+ and Cl^- in the protoplasm leads to ionic imbalance and induces ion-specific effects in enzymes, proteins and membranes (Arshi *et al.,* 2002, 2004; Qureshi *et al.,* 2013). In the NaCl-treated plants, oxidative stress could stem from a decreased stomatal conductance in response to osmotic imbalance and reduced leaf-water potential (Qureshi *et al.,* 2005), ultimately leading to a decrease in photosynthesis and biomass accumulation (Yamaguchi & Blumwald, 2005). Even the secondary-metabolite production is hampered (Iqbal, 2013; Qureshi *et al.,* 2013).

Like some earlier reports on cucumber (Kaya *et al.,* 2003; Tiwari *et al.,* 2010), tomato (Agong *et al.,* 2003) and soybean (Arshi *et al.,* 2010a), the present investigation also showed reduced contents of photosynthetic pigments with increase in NaCl concentration. Among the cultivars, the highest chlorophyll content was recorded in Dosha and the lowest in Hachalu. The decrease in chl content was greater at the highest salinity level. Reduction in chl content may occur due to increase in degradation or decrease in synthesis of chlorophyll (Santos, 2004). Salinity stress reduces the number of chloroplasts and brings about decomposition of the thylakoid and plastid membranes. Chloroplast decomposition may increase the activity of chlorophyllase enzyme and decrease the amount of chlorophyll (Fang *et al.,* 1998). Reduction in chl content under salinity is also attributed to destruction of chlorophyll molecule and instability of pigment-protein complex (Jaleel *et al.,* 2008). Insufficient chlorophyll content might lead to malfunction of the photosystem, causing a fall in the total CO_2 fixation (Woodward & Bennett, 2005; Bashir *et al.,* 2015). This has a direct bearing on growth and productivity of the plant.

Photochemical efficiency of PSII (*Fv/Fm*) can be used as a criterion for evaluating plant performance under stressful conditions (Husen *et al.,* 2014, 2017; Getnet *et al.,* 2015; Oukarroum *et al.,* 2015) and is thus important to determine seedling-stock quality (Husen *et al.,* 2004a,b; Kalaji *et al.,* 2011; Husen, 2009, 2013). In our study, decline of the *Fv/Fm*value under NaCl stress suggests that salinity affected some process related to the photochemistry of photosynthesis. Reduction in *Fv/Fm*under stressful condition is not uncommon (Kalaji *et al.,* 2011; Li *et al.,* 2013; Husen *et al.,* 2014, 2016; Embiale *et al.,* 2016) and may be positively correlated to a decrease in different photosynthetic parameters and biomass production. Dosha had a higher *Fv/Fm*value than the other cultivars. Low salinity stress did not cause a significant difference from the control, thus suggesting a non-significant impact on PSII-reaction centers. In barley plants, negative influence of salinity on PSII activity was dependent on stress duration and the cultivar used (Kalaji *et al.,* 2011). The decline in net photosynthetic rate*(Pn),* stomatal conductance (*gs)* and transpiration rate (*E)* values was dose-dependent in all the cultivars tested. Cultivar Dohsa was relatively superior in terms of *Pn* and *E*. The decline in *Pn* can be attributed to stomatal factors. CO_2 concentration in chloroplasts may decrease because of a reduction in stomatal conductance, despite a stable CO_2 concentration in the substomatal spaces (Tourneux & Peltier, 1995). In the salt-affected plants, closing of stomata and a decrease in quantity and/or activity of Rubisco may also be the cause of decline in *Pn* (Heuer & Plaut, 1989; Arshi *et al.,* 2004, 2006). Parida *et al.* (2004) reported a slight increase in *Pn* at low salinity but a decrease at high salinity levels, although *gs* remained unchanged at low salinity and decreased at high salinity. The limited rise of *Pn* under low stress could well be a case of hormesis (Melki & Dahmani, 2009; Aref *et al.,* 2015), which involves causation of positive biological response to low doses of stress through activation of repair mechanism, leading to improved immunity level of the biological system involved.

In conclusion, salinity stress hampers the vegetative growth of *V. faba* cultivars by affecting plant biometry, water status, photosynthetic pigments, gaseous exchange and photosynthetic efficiency. Of the three cultivars tested, Dosha was more tolerant to salt-stress-induced damage than others.

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