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Optimization of Paclobutrazol Dose for Foliar and Drenching Applications under Water Deficit Stress in Chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea (*Cicer arietinum* L.) is the fourth most important pulse crop rich in protein by virtue of N_2 fixation. Water deficit is a major limitation in chickpea production. Paclobutrazol (PBZ), a potential triazole, has been reported to provide plant protection against abiotic stresses. Therefore, an experiment was conducted using *kabuli* chickpea variety Pusa 1108 (sensitive to water deficit) to identify its optimum dose for foliar and drenching application. Water deficit stress was imposed by withholding the water at vegetative stage. Just prior to water stress treatment, plants were treated exogenously with varying dose paclobutrazol for foliar (0, 30, 60, 90, 120 and 150 mg l^{-1}) and drenching (0, 60, 120, 180, 240 and 300 mg l^{-1}) applications. Maximum enhancement of relative water content (RWC), membrane stability index (MSI), photosynthetic rate (P_N), chlorophyll$_a$ (chl_a), chlorophyll$_b$ (chl_b) and total chlorophyll during water deficit stress and after recovery were obtained with the foliar application of PBZ @ 60 mg l^{-1} while for PBZ drenching @ 120 mg l^{-1}. On an average, in general based on curve fitting of under taken physiological responses of chickpea to PBZ, optimum dose of PBZ foliar application was estimated 67.5 mg l^{-1} while for PBZ drenching 127 mg l^{-1}.

Keywords: Chickpea, optimum dose, paclobutrazol, photosynthesis, water deficit stress

1. Introduction

Chickpea is the most important crop representing about 27% of the land area under pulse, which contributes 33% of the total pulse production in india. In India, chickpea is grown over an area of 8.52 mha with production of 8.83 mt and average productivity of 1036 kg ha^{-1} (Anonymous, 2014). Water deficit stress is one of the major abiotic stresses which adversely affect crop growth and yield. Water deficit affects many morphological features and physiological processes associated with plant growth and development in chickpea (Toker and Cagirgan, 1998). These changes include reduction of relative water content (RWC), diminished leaf water potential and turgor loss, closure of stomata and a decrease of cell enlargement and plant growth. Water deficit stress reduces plant growth by affecting photosynthesis, respiration, membrane stability index (MSI) and nutrient metabolism (Jaleel et al., 2007). Cell membrane stability has been used as a water deficit stress tolerance test (Agarie et al., 1995) and electrolyte leakage showed an increase with increasing water deficit (Gopi et aol., 2007). Water deficit stress also inhibits the photosynthesis of plants by affecting chlorophyll components and damaging the photosynthetic apparatus (Iturbe-Ormaetxe et al., 1998).

Since chickpea is traditionally cultivated in marginal and rainfed areas (Rao et al., 2002), therefore, drought is a major constraint in chickpea production that causes 40–50% reductions in yield globally (Ahmad et al., 2005).Therefore, an alternative approach that attracts more attention in this direction is the application of some external plant growth regulating molecules which can be used to achieve the stress tolerance in crops without compromising yield under water deficit stress. Paclobutrazol has innate ability to induce abiotic tolerance by increasing antioxidant enzymes and molecules in oxidative stressed plants. Paclobutrazol has been proved as an agent in stress amelioration in medicinal plants (Jaleel et al., 2007). PBZ has been used to provide plant protection against abiotic stresses viz chilling (Lin et al., 2006), water deficit stress (Zhu et al., 2004), flooding (Lin et al., 2006) and salinity (Kishor et al., 2009). Paclobutrazol was also reported to induce water deficit stress tolerance in ground nut (Sankar et al., 2007). Though chickpea is grown in rainfed areas despite that to date, not a single report is available on the PBZ induced water stress tolerance in chickpea. Therefore, a preliminary study was carried out with an objective to identify PBZ optimum dose for foliar and drenching application in chickpea.

2. Materials and Methods

2.1. Plant material and growth conditions

Present study was carried out in pot culture inside the net house of Division of Plant Physiology, IARI, New Delhi-110012 (latitude of 28°N and longitude of 77°E, and about 250 m (Above mean sea level) to identify optimum dose of paclobutrazol for foliar and drenching applications. Seed of *Kabuli* chickpea variety Pusa 1108 was obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Sowing was done in 12 inch diameter earthen pots filled with clay loam soil and farmyard manure in 4:1 ratio during winter season inside net house of Division of Plant Physiology, IARI, and New Delhi. All recommended agronomic practices were followed to raise the healthy crop plants. Recommended basal dose of fertilizers in the form of urea, SSP and MOP were applied to raise the healthy crop.

2.2. Experimental details and sample collection

A pot experiment was conducted in complete randomized design using with three replications. Water stress was imposed at vegetative stage in chickpea variety by restricting water supply until the appearance of severe symptoms of wilting. Before imposition of water stress plants were treated exogenously with varying dose paclobutrazol (Sigma-Aldrich) for foliar (0, 30, 60, 90, 120 and 150 mg l^{-1}) and drenching (0, 60, 120, 180, 240 and 300 mg l^{-1}) applications. Volume of PBZ solutions prepared with varying doses was kept 50 and 100 ml pot^{-1} for foliar and drenching applications respectively. Plant samples were taken on 12th days after withholding water during imposed water deficit stress. After sampling, water deficit stress was terminated and further sampling was done during recovery period i.e. on 6th day after water stress termination. Observations were recorded on photosynthetic pigments, photosynthesis, MSI, RWC. Based on the physiological response data of both foliar and drenching applications optimum dose were identified by curve fitting.

2.3. Soil moisture content

Soil moisture content was estimated periodically during imposed water stress treatment at vegetative stage by gravimetric method. Soil sample was taken from pot at 10 cm depth using auger. For each sample 100 g fresh weight of soil was recorded using a weighing balance. Then the sample was kept in the oven at 105 °C, and dried till constant weight was achieved. The soil moisture content was calculated by the following formula (Faulkner et al., 1989) and expressed in percentage (%). Fully saturated soil at 100% capacity (FC) had 30% moisture. By using this relation of field capacity with soil moisture FC (%) was estimated at different water deficit stressed period (Figure 1).

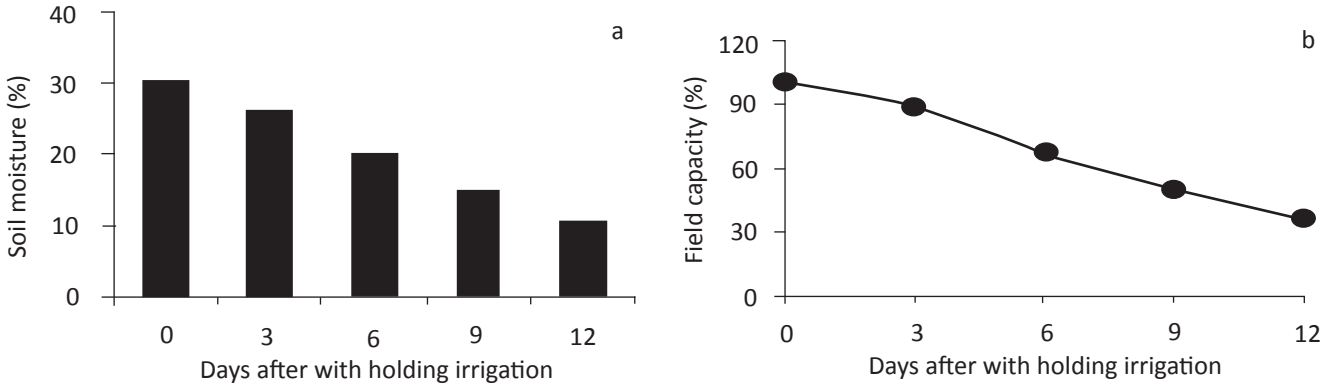

Figure 1: Soil moisture content (a) and field capacity (b) recorded during the course of imposed water deficit stresscondition

2.4. Relative water content

Leaf relative water content (RWC) was estimated by recording the turgid weight of 1 g fresh leaf samples by keeping in water for 4 h, followed by drying in hot air oven at 70 °C till constant weight was achieved (Weatherley, 1950).

RWC=[(Fresh wt.–Dry wt.)/(Turgid wt.–Dry wt.)]×100

2.5. Membrane stability index

Membrane stability index (MSI) was estimated according to the method described by Premachandra et al. (1990). For estimation of membrane stability index 100 mg leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40 °C for 30 min in a water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C_1). Second set was boiled at 100 °C in a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C_2). Membrane stability index (MSI) was calculated as:

$MSI=[1-(C_1/C_2)]\times100$

2.6. Photosynthetic rate

Rate of photosynthesis was measured using portable Infrared Gas Analyzer (IRGA), model LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA). Data on rate of photosynthesis (µmol CO_2 m^{-2} s^{-1}) were recorded on clear sunny day between 10 to 11.30 am by providing artificial light source of intensity 1200 µmol m^{-2} s^{-1} during water deficit stress and after water deficit stress termination (during recovery). Fourth fully expanded leaf from top was used for the measurement of photosynthesis using fixed exposed area of leaf.

2.7. Chlorophyll content

Chlorophylls content were estimated as per the method described by Hiscox and Israelstam (1979). The procedure for estimation of chlorophyll content in plants is based on the absorption of light by chlorophyll extracts prepared by incubating the leaf tissues in dimethyl sulfoxide (DMSO). DMSO renders plasmalemma permeable thereby, causing the leaching of the pigments. Fifty mg fresh leaf samples were added to the test tubes containing 10 ml DMSO. Tubes were kept in dark for 4 h at 65 °C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663 and 645 nm using DMSO as blank and was expressed as mg g^{-1} dry wt. The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645) was determined for chlorophylls content. Chlorophyll a, chlorophyll b and total chlorophyll content were estimated using the formula given by Arnon (1949).

Chlorophyll 'a' = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/W \times 1000$

Chlorophyll 'b' = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/W \times 1000$

Total chlorophyll=$(20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1000$

Where,

A_{663}=Absorbance values at 663 nm

A_{645}=Absorbance values at 645 nm

W=Weight of the sample in g

V= Volume of the solvent used (ml)

3. Results and Discussion

For the identification of optimum dose of PBZ for foliar application varying doses of PBZ (0, 30, 60, 90, 120 and 150 mg l^{-1}) were applied on plants at vegetative stage. All physiological parameters (RWC, MSI, photosynthetic rate and photosynthetic pigments) were increased with increasing PBZ dose upto 60 mg l^{-1} and decreased thereafter at its higher doses during imposed water deficit and after water stress termination (during recovery) in both the varieties. Based on curve fitting, optimum response of chickpea in term of leaf RWC was obtained at PBZ dose of 72 mg l^{-1} under water deficit stress as well as during recovery (Table 1 and Figure 2a). Similarly based on MSI values, optimum PBZ dose for foliar application was estimated 60 mg l^{-1} under water deficit (Figure 2c). While after water deficit stress termination during recovery optimum PBZ foliar dose was 90 mg l^{-1} (Figure 2c). Based on P_N values optimum PBZ foliar dose was found 90 mg l^{-1} under water deficit, while during recovery plants had 60 mg l^{-1} PBZ optimum dose (Figure 2e). Similarly, based on leaf chla and chlb contents values optimum PBZ foliar application dose was estimated 60 mg l^{-1} both under water deficit as well as during recovery (Figure 3a and Figure 3c). However, total chlorophyll contents indicated 66 mg l^{-1} PBZ optimum dose for foliar application under water deficit and 60 mg l^{-1} during recovery (Figure 3e). On an average, in general based on curve fitting of under taken physiological responses of chickpea to PBZ, optimum dose of PBZ foliar application was estimated 67.5 mg l^{-1} (Table 1).

Further, for optimizing PBZ dose for drenching, varying dose of PBZ (0, 60, 120, 180, 240 and 300 mg l^{-1}) were used for drenching before imposing water deficit stress. PBZ drenching treatments also increased RWC, MSI, P_N, chl_a, chl_b and total chlorophyll with increasing dose of PBZ up to 120 mg l^{-1} and decreased at its higher dose during imposed water deficit and recovery in chickpea. Based on curve fitting using leaf RWC values optimum PBZ dose for drenching was estimated 132 mg l^{-1} under water deficit and 120 mg l^{-1} during recovery (Fig. 2b). Similarly, based on curve fitting MSI, P_N, chl_b and total chlorophyll values also indicated 120 mg l^{-1} PBZ optimum dose for drenching application under water deficit as well as during recovery (Figure 2 (d and f); Figure 3 (d and f). However, chl_a content indicated higher optimum PBZ dose for drenching i.e. 132 mg l^{-1} under water deficit stress and 180 mg l^{-1} during recovery after water deficit stress termination (Figure 3b). On an average, based on curve fitting using physiological responses values of chickpea to PBZ, optimum dose of PBZ drenching application was estimated 127 mg l^{-1} (Table 1).

Based on recorded RWC values with varying PBZ doses, estimated optimum PBZ dose for foliar and drenching applications under water deficit stress condition were 72 mg l^{-1}, 132 mg l^{-1} similarly, after water deficit termination during recovery PBZ optimum doses were 72 mg l^{-1}, 120 mg l^{-1} for foliar and drenching application respectively. RWC was decreased under water deficit condition while PBZ applications improved leaf RWC in chickpea variety. Reduction in RWC of leaves was observed in plants exposed to water deficit conditions. There are reports that PBZ treated plants of *Curcuma alismatifolia* Gagnep (Jungklang and Saengnil, 2012) and cucumber seedling (Baninasab and and Ghobadi, 2011) had higher value of RWC. Paclobutrazol has been reported to enhance water retention and thus increased survival in wheat (Aly and Lathif, 2011) and barley (Rady and Gaballah, 2012) by accelerating stomatal response and reducing transpiration rate under water deficit condition.

Similarly, based on MSI values estimated optimum PBZ dose for foliar and drenching applications under water deficit stress condition were 60 mg l^{-1} and 120 mg l^{-1} respectively while during recovery optimum PBZ doses for foliar and drenching applications were calculated 90 mg l^{-1}, 120 mg l^{-1}. MSI was reduced under water deficit. The inhibition of electrolyte leakage indicates ability of PBZ in maintaining the membrane integrity (Fletcher et al., 2000) of plants. PBZ altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane (Burden et al., 1987). In addition, up-regulation of stress protective biomolecules in PBZ treated plants enhances the capacity to limit the damage caused by species of reactive oxygen (Fletcher and Hofstra, 1990). PBZ has been reported to reduce electrolyte leakage in wheat

Table1: Optimization of PBZ dose for foliar and drenching application under water deficit condition and after water deficit stress termination during recovery

Sl. Nl.	Parameters used for optimizing PBZ dose	Under water deficit stress			After water deficit stress termination (during recovery)		
		Regression equation	R^2	Estimated optimum dose (mg l^{-1})	Regression equation	R^2	Estimated optimum dose (mg l^{-1})
				Foliar Application			
1.	RWC	$y=-0.001x^2 + 0.177x+51.37$	0.850	72	$y=-0.000x^2+0.073x+81.17$	0.922	72
2.	MSI	$y=-0.000x^2+0.042x +72.56$	0.790	60	$y =-0.000x^2+0.106x+65.24$	0.844	90
3.	P_N	$y=-0.000x^2+0.132x+ 9.334$	0.920	90	$y=-0.000x^2+0.059x+14.84$	0.823	60
4.	Chl_a	$y=-5E-05x^2+0.005x+0.946$	0.718	60	$y=-8E-05x^2+0.010x+1.796$	0.837	60
5.	Chl_b	$y=-1E-05x^2+0.001x+0.252$	0.959	60	$y=-1E-05x^2+0.001x+0.202$	0.943	60
6.	Total Chl	$y=-5E-05x^2+0.005x+0.946$	0.718	66	$y=-5E-05x^2+0.006x+2.155$	0.833	60
	Mean			68	Mean		67
Grand mean= (Mean under water deficit stress+mean after water deficit stress termination)/2 =							67.5
Drenching application							
1.	RWC	$y=-0.000x^2+0.168x+ 48.72$	0.795	132	$y=-0.000x^2+0.079x+77.13$	0.846	120
2.	MSI	$y=-0.000x^2+0.045x+ 68.34$	0.715	120	$y=-0.000x^2+0.038x+68.93$	0.767	120
3.	P_N	$y=-0.000x^2+0.070x +11.5$	0.745	120	$y=-0.000x^2+0.131x+12.47$	0.813	120
4.	Chl_a	$y=-2E-05x^2+0.005x+ 1.073$	0.701	132	$y=-1E-05x^2+0.004x+1.294$	0.775	180
5.	Chl_b	$y=-1E-05x^2+0.003x+ 0.372$	0.970	120	$y=-3E-06x^2+0.000x+0.199$	0.928	120
6.	Total Chl	$y=-3E-05x^2+0.008x+ 1.486$	0.976	120	$y=-2E-05x^2+0.005x+1.474$	0.609	120
	Mean			124	Mean		130
Grand mean= (Mean under water deficit stress+Mean after water deficit stress termination)/2 =							127

(Aly and Latif, 2011) and horse chestnut (Percival and Noviss, 2008) during water deficit stress.

Based on P_N, chickpea plants performed better under optimum PBZ doses for foliar and drenching application during water deficit stress condition were 90 mg l^{-1}, 120 mg l^{-1} and after water deficit termination, showed response to optimum doses of 60 mg l^{-1}, 120 mg l^{-1} for foliar and drenching applications respectively. PBZ enhanced the P_N in chickpea plants. Paclobutrazol has also been reported to enhance net photosynthesis rate in triticale (Berova and Zlatev, 2003), *Setaria italica* (Bisht et al., 2007) and horse chestnut (Percival and Noviss, 2008). The higher chlorophyll content in the leaves, leading to higher photosynthesis in the triazole treated plants (Feng et al., 2003). On the other hands, PBZ also increased activity of RUBP-carboxylase and thus rate of photosynthesis in peanut (Yan and Pan, 1992). Further, Paclobutrazol was reported to significantly enhance zeatin and zeatin riboside (Zhu et al., 2004) and cytokinins protect photosynthetic machinery.

Estimated PBZ optimum doses based on chl_a, chl_b, and total chlorophylls values for foliar were 60 mg l^{-1}, 60 mg l^{-1} and 66 mg l^{-1} respectively during water deficit stress. Similarly, estimated

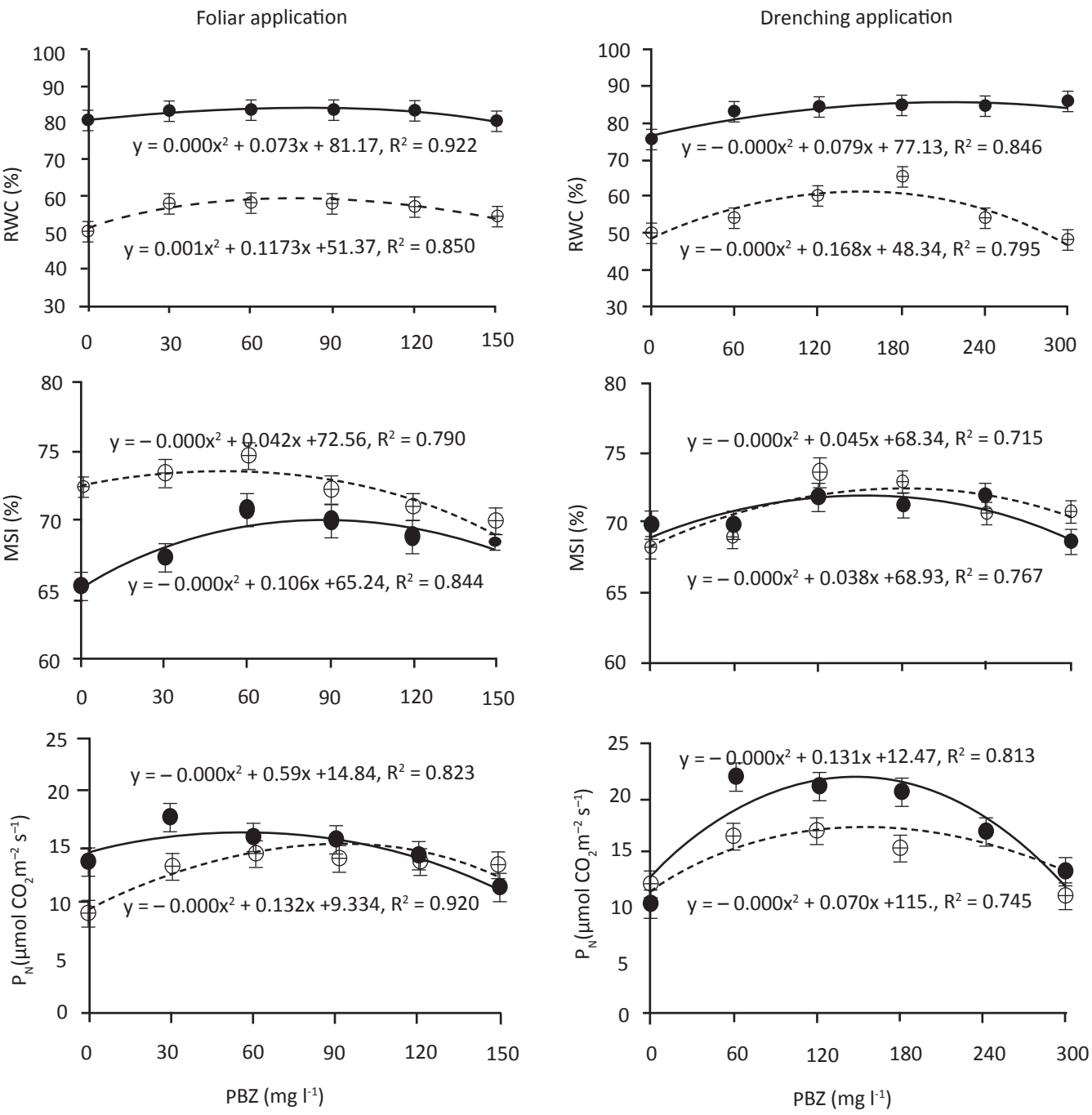

Figure 2: Relative water content, membrane stability index and photosynthesis rate response of chickpea plants to foliar and drenching application of PBZ under water deficit stress (-○○○) and during recovery (-●●●) .
(a) RWC response to PBZ foliar application under water deficit stress and during recovery, b) RWC response to PBZ drenching application under water deficit stress and during recovery, c) MSI response to PBZ foliar application under water deficit stress and during recovery, d) MSI response to PBZ drenching application under water deficit stress and during recovery, e) PN response to PBZ foliar application under water deficit stress and during recovery f) P_N response to PBZ drenching application under water deficit stress and during recovery).

PBZ optimum dose based on chl_a, chl_b, and total chlorophylls values for drenching application during water deficit stress condition were 132 mg l^{-1}, 120 mg l^{-1}, 120 mg l^{-1} respectively. During recovery also calculated optimum dose based on chl_a, chlorophylls values (chl_b and total chlorophylls) for foliar were 60 mg l^{-1}. Similarly, PBZ optimum dose based on chl_a, chl_b and total chlorophylls values for drenching application were 180 mg l^{-1}, 120 mg l^{-1} and 120 mg l^{-1} respectively. PBZ applications

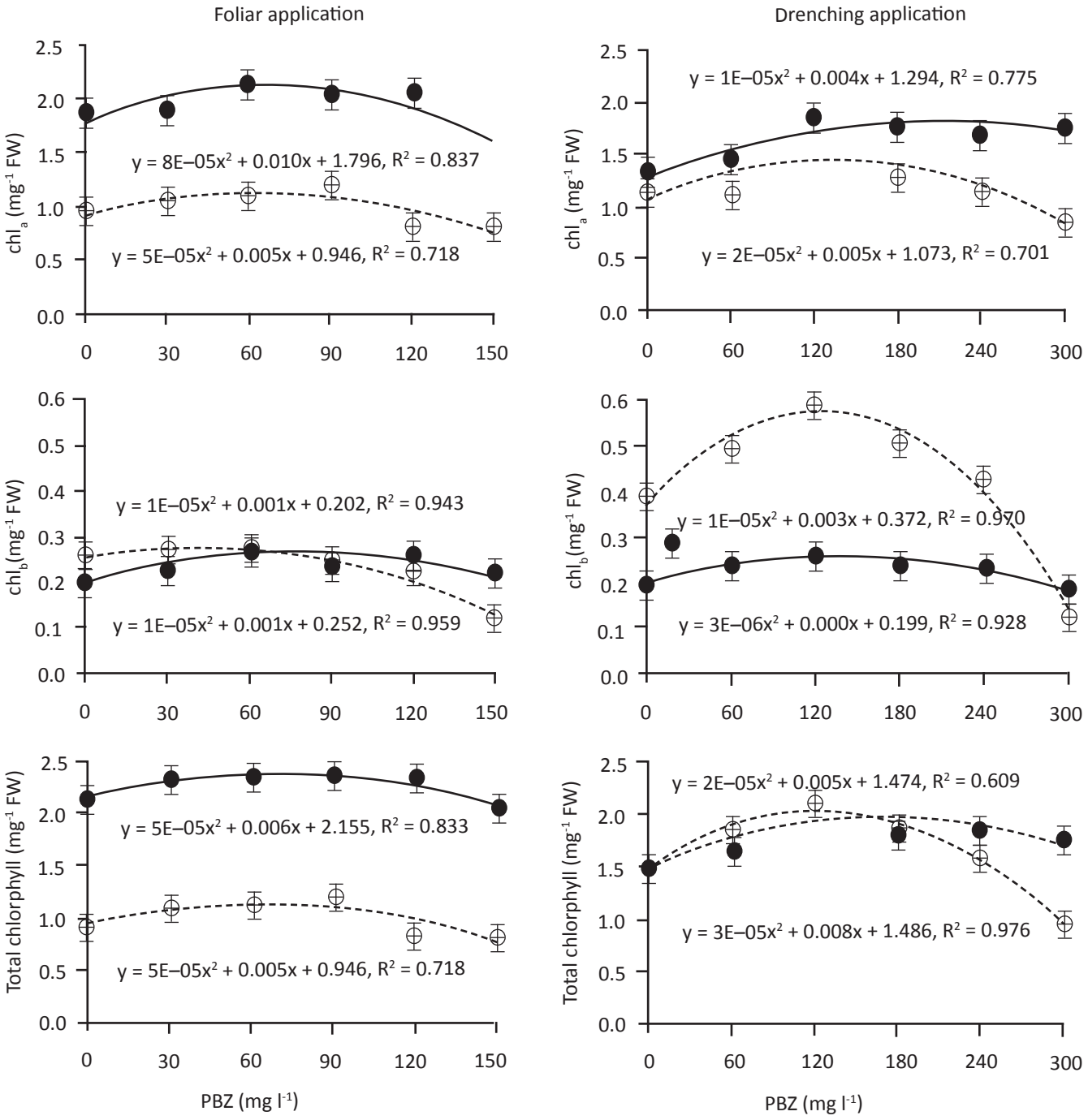

Figure 3: Chlorophyll a (chl$_a$), chlorophyll b (chl$_b$) and total chlorophyll response of chickpea plants to foliar and drenching application of PBZ under water deficit stress and during recovery. (a) chl$_a$ response to PBZ foliar application under water deficit stress and during recovery, (b) chl$_a$ response to PBZ drenching application under water deficit stress and during recovery, (c) chl$_b$ response to PBZ foliar application under water deficit stress and during recovery, (d) chl$_b$ response of chickpea plants to PBZ drenching application under water deficit stress and during recovery, (e) Total chlorophyll response to PBZ foliar application under water deficit stress and during recovery (f) Total chlorophyll response to PBZ drenching application under water deficit stress and during recovery).

improved or maintained higher level of photosynthetic pigments under water stressed condition in chickpea variety by increasing level of cytokinins and antioxidant activity. PBZ application has been reported to increase level of pigments in cucumber (Baninasab and Ghobadi, 2011) and wheat (Nouriyani et al., 2012). The decrease in chlorophyll

under water deficit stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). A reduction in photosynthetic pigments content was reported in water stressed cotton (Massacci et al., 2008), horse chestnut and *Catharanthus roseus* (Jaleel et al., 2007). PBZ is known to block the production of gibberellins, resulting in utilization of intermediates of gibberellin synthesis to facilitate increased phytyl production (Chaney, 2003) which should have resulted in higher level of chlorophylls and dark green leaf colour as was observed during the present study.

In general, RWC, MSI, photosynthesis rate, chl_a, chl_b, and total chlorophylls increased with increasing dose of PBZ used for foliar and drenching applications during water deficit stress condition as well as after water deficit termination during recovery. On an average, in general based on curve fitting of RWC, MSI, photosynthetic rate, chl_a, chl_b and total chlorophyll responses of chickpea to PBZ, optimum dose of PBZ during water deficit stress and after water deficit stress termination (during recovery), were estimated 67.5 mg l^{-1} and 127 mg l^{-1} for foliar and drenching applications respectively.

Maximum enhancement of RWC, MSI, P_N, chl_a, chl_b and total chl during water deficit stress and after recovery were obtained with the foliar application of PBZ @ 60 mg l^{-1} while for PBZ drenching @ 120 mg l^{-1} However at higher doses there was either stagnation or reduction in the values of aforementioned parameters probably due to the meatabolic imbalances by vigorously altering the level of endogenous hormones at higher dose of PBZ. Similarly, higher dose of PBZ has also been reported inhibitory in tomato (Rahman et al., 1989).

4. Conclusion

Maximum enhancement of RWC, MSI, photosynthetic rate, chla, chlb and total chlorophyll were recorded with the foliar application of PBZ @ 60 mg l^{-1} and for PBZ drenching @ 120 mg l^{-1}. On an average, based on curve fitting of physiological responses, optimum dose of PBZ foliar application was estimated 67.5 mg l^{-1} while for PBZ drenching 127 mg l^{-1}. Physiological performances were improved with the applications of PBZ. PBZ treated plants showed higher level of RWC by altering stomatal response. Further, PBZ treatments increased chlorophylls content by inducing alteration in hormonal biosynthesis pathways; thus enhanced the rate of photosynthesis.

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