Study the Effects of Fulvic Acid on Physiological Traits of *Citrus Unshu* under Salt Stress

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***Abstract*—**The effects of soil application of fulvic acid on some physiological traits of *Citrus unshu* grown at various salt concentrations were examined. Sodium chloride was added to the soil to obtain 25 and 50 mM saline conditions. The liquid fulvic acid was put into the soil pots three times on 15,30 and 45th day after Bud emerged. The application doses of fulvic acid were 0, 50 and 100 m g/l and the NaCl doses were 0, 25, and 50 mM and Each application consists of four replications. The experiment was conducted in pot experiment under controlled conditions in completely randomized factorial design. Finding of this research showed that by increasing salinity levels, amylytic activity and relative humidity increased, but the amounts of chlorophyll b and proline of leaf decreased significantly. The usage of fulvic acid were only effective to adjust of salinity effects on the amounts of proline and chlorophyll a at the level of 50 Mm. Also, the effects of fulvic acid on amylase and chlorophyll b of leaf were changeable, so that some levels of fulvic acid increased and some levels decreased the measurements of these physiological traits.

***Keywords*—** Fulvic acid, *Citrus unshu*, Physiological Traitss, Salt stress.

I. Introduction

CITRUS unshu is native to South Asia. *Citrus unshu* is commercially cropped in many countries such as Usa, Spain, China, Iran and Greece (Ferguson, 1999). Salt stress is characterised by high amounts of Na +, Mg +2, Ca +2, Cl –, HCO 3–, SO 4–2, and B ions which have negative effects on the plant growth. Generally, NaCl causes salt stress in nature. The adverse effects of salinity mainly involve two aspects: increased osmotic potential stress, and possible toxic effects of excessive ions. Physiological responses to salinity include shoot dehydration, lowered osmotic potential, and/or a loss of turgor potential, resulting in reduced growth rate. fulvic acids may promote the growth and development of plants by improving the nutrient uptake (Clapp et al., 2001; Varanini and Pinton, 2001). On the other hand, increasing the growth status of plants (Sanz et al., 2004; Razavi and Parvar, 2007).

In many studies, organic acids were reported to improve the physiological traits (Ahmad et al.,2010). Briefly, high salt concentrations in the soil reduce the physiological activities of plants and negatively affects the growth and development (Khaled and Hassan, 2011). However, there is a lack of research regarding fulvic acid application and its effects on Citrus unshu salinity tolerance. Therefore, The objective of this study was to compare the effect of fulvic acid on the physiological traits of Citrus unshu under Salinity condition

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II. Material And Methods

The soil used in this study was collected from 0–30 cm depth of the field located in Amol. The planting dates were on 15th March. Some physical and chemical properties of the soil were determined and As a basal fertilization, the fertilizer crystalon comprised of nitrogen, phosphorus, potassium, Fe, Mn and Cu were applied to the pots before planting. The experiment was conducted in Pot experiment under controlled conditions in completely randomized factorial design with three soil application doses of fulvic acid 0, 50, and 100 mg/l and three NaCl doses 0, 25, and 50 mM and each application consists of four replications. All pots were irrigated with distilled water during the experiment. The soil applied fulvic acid was prepared from humax company (United states) .Air-dried soil samples were passed through 4 mm sieve. After two months vegetation period, the plants were discarded and dried at 60°C and samples were wet digested by using HNO 3 + HCIO 4 (4:1). The amounts of proline (Bates, 1973), chlorophyll a and chlorophyll b (Jensen, 1978) and amylytic activity (Bernfeld, 1965) by spectrophotometer and relative humidity by oven (AOAC,1990) were determined. The statistical analysis was performed using SAS, MSTATC statistical softwares, and mean values were grouped with Duncan multiple range test ($P< 0.05$).

III. Results

A. Amylase

According to the analysis of variance, salinity was significant at Probablity level of 0.05 and spraying and interaction effects between salt and spraying were found statistically significant ($P<0.01$)(table1). The most measurement of amylytic activity by the treatment of S25F100 (0.95 unit/mg) was obtained.

B. Chlorophyll a

According to the analysis of variance, spraying was significant at Probablity level of 0.05 and interaction effects between salt and spraying was found statistically significant

(P<0.01)(table1). The most measurement of Chlorophyll a by the treatment of S0Control (0.06 mg/g) was obtained.

C. Chlorophyll b

According to the analysis of variance, salinity was significant and spraying at Probablity level of 0.01 and interaction effects between salt and spraying was found statistically significant (P<0.05)(table1). The most measurement of Chlorophyll b by the treatment of S0F100 (0.04 mg/g) was obtained was obtained.

D. Proline

According to the analysis of variance, salinity, spraying and interaction effects between salt and spraying were found statistically significant (P<0.01)(table1). The least measurement of proline by the treatment of S50Control (0.75 mg/g) was obtained.

E. Relative humidity (RWC)

According to the analysis of variance, salinity was significant at Probablity level of 0.05 and spraying and interaction effects between salt and spraying were found statistically significant (P<0.01)(table1). The least measurement of RWC by the treatment of S0F50 (27.95%) was obtained.

IV. Discussion

A. Amylase

In saline soil, the activity of amylase is particularly low and the plants grown in these soils often show deficiencies of reduced sugar. These Results corroborate findings of Ahmad (2010), Farhoodi et al., (2012), Hoque (2008), Hafez et al.,(2004), Bianco & Defez (2009) and Nounjan & Theerakulpisut (2012).

B. Chlorophyll a

One negative effect of increasing salinity is decreased leaf chlorophyll a and, consequently, decreased phytosynthetic activity. These Results corroborate findings of Silva et al., (2008), Celik & Cimen (2012), Sumalee (2011), Hare (2002), Qureshi (2005), Foolad & Ashraf (2007) and Ranjbar (2000).

C. Chlorophyll b

Salinity increases the measurement of Chlorophyll b and this is a protecting mechanism. These Results corroborate findings of Clapp et al., (2001), Desingh & kanagaraj (2007), Anjum (2008), Karimi (2009), Chen & Aviad (2004), Das et al., (2002) and Yan (2011).

D. Proline

Many laboratory and glasshouse studies have shown that salinity increase osmolites such as proline accumulation in plants. These Results corroborate findings of Shiwani et al., (2010), vilchez (2000), Yuan (2009), Karimi et al.,(2009), Kumar et al., (2003), Choudhary et al., (2009) and Widodo (2008).

E. Relative humidity

A high salt content decreases the osmotic potential of the soil water and this reduces the availability of the soil water for plants, and consequently reduces the relative humidity. These Results corroborate findings of Shiwani et al., (2010), Nasirkhan et al., (2007), Anjum (2008), Al-Yassin (2004), khan et al., (2004) Tantawy (2009) and Guerfel (2008).

V. Conclusion

Regarding our finding, fulvic acids are effective or ineffective on physiological traits. This study indicated that the application doses are important for deriving benefit from fulvic acids under salinity conditions. However, there are not many researches into fulvic acid application and their effects on Citrus unshu salinity tolerance. Therefore, more researchs is necessary to explain the positive effects of fulvic acid on Citrus unshu plants under salt stress.

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TABLE I
ANALYSIS OF VARIANCE OF FULVIC ACID ON PHYSIOLOGICAL TRAITS UNDER SALT STRESS

Variation Source	DF	RWC	Proline	Chl a	Chl b	Amylase
salt	2	60.23*	8.89**	0.00007693^{ns}	0.0005029**	0.026*
spray	4	79.31**	1.50**	0.00029538*	0.00045075**	0.134**
salt*spray	8	73.88**	3.06**	0.00040596**	0.00014658*	0.118**
Error	45	18.66	0.0016	0.00006671	0.0000666	0.0069
CV%		11.81	2.73	18.48	32.86	15

Ns,*,**Nonsignificant or significant at P = 0.05, 0.01 respectively.

TABLE II
EFFECTS OF FULVIC ACID ON PHYSIOLOGICAL TRAITS UNDER SALINITY

Treatment	RWC(%)	Prolin (mg·g/fw)	Chl b (mg·g/1)	Chl a (mg·g/fw)	Amylase (unit.mg/fw)
S0CONTR	41.01de	1.46i	0.0390c	0.0701a	0.76d
S0F50	27.95f	1.25j	0.0363b	0.0494e	0.49i
S0F100	41.68ab	2.31d	0.0398a	0.0587b	0.91b
S25CONTR	31.65e	4b	0.0268f	0.0416h	0.69e
S25F50	37.43 bcd	2e	0.0209j	0.0343j	0.48k
S25F100	36.30 cde	1.05k	0.0226g	0.0398i	0.96a
S50CONTR	42.46a	0.75m	0.0146m	0.0404i	0.50l
S50F50	41.57ab	1.50f	0.0225h	0.0488f	0.58g
S50F100	39.44abc	1.05k	0.0198l	0.0569c	0.81c