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Changes in soluble and insoluble carbohydrates of safflower (*Carthamus tinctorius* L.) influenced by supplemental irrigation and super absorbent polymer

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Key words: Soluble carbohydrate, Insoluble carbohydrates, Safflower, Yield.

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Abstract

The present investigation studied the effects of supplemental irrigation, the application of Super Absorbent Polymer (SAP) on seed yield, and the physiological characteristics relevant to water stress in leaves and roots of safflower. Supplemental irrigation was implemented at 4 levels including dry-farming; supplemental irrigation was implemented at late vegetative, flowering, and filling of seeds phases as main factors. Super absorbent polymer (SAP) at two levels (Control and 200kg/hectares) and 6 safflower varieties were used in winter cultivation in the factorial form as sub-factors. The results indicated that supplemental irrigation can prevent a reduction in seed yield by improving physiological parameters like soluble and insoluble carbohydrates. At the flowering stage, supplemental irrigation increased seed yield an average of 35% compared with dry-farming, thereby producing the maximum rate of seed yield in both test years. soluble carbohydrate rate was reduced under the influence of supplemental irrigation, but insoluble carbohydrates concentration was increased. Leaves and roots varied similarly in respect to any parameters, but their concentrations were different. Employing SAP can affect all studied parameters and lead toward a reduction of drought stress. It is suggested that supplemental irrigation together with an application of SAP be used for the safflower in its flowering phase in order to reduce drought stress loss and to increase yield in dry-farmed cultivations.

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Introduction

Safflower is a strongly tap rooted annual plant belonging to the Asteraceae family and is native to the Middle East. It is resistant to saline conditions (Francois and Berstein, 1964), to water stress (Bassiri *et al.,* 1977), and can reach the deep-lying water (Weiss, 2000). In addition, the importance of oil seed crops such as safflower has increased in recent years, especially with the growing interest in the production of biofuels (Dordas and Sioulas, 2008). Safflower (*Carthamus tinctorious* L.) is a temperate zone plant grown in arid and semiarid regions of world (Mcpherson *et al.,* 2004). Kar *et al.* (2007) reported that there was a need for irrigation of safflower in addition to winter and spring precipitations. Supplemental irrigation (SI) is defined as the application of a limited amount of water to the crop when rainfall fails to provide sufficient water for plant growth to increase and stabilize yields. The additional amount of water alone is inadequate for crop production (Oweis, 1997). Hence, the essential characteristic of SI is the supplemental nature of rainfall and irrigation. However, SI is usually practiced in the wetter part of the dry areas (300–600 mm annual rainfall) (ICARDA, 2003; Oweis and Hachum, 2004). All field crops response differently in different phonological stages to changing water status of the soil under deficit irrigation, which means that plants are more sensitive to water deficit at one or more stages than at the other stages (Kirda, 2002). Available limited researches in around the world on safflower production under irrigated conditions revealed that it was a sensitive crop to water (Quiroga *et al.,* 2001; Bassil and Kaffka, 2002a,b) and moderately tolerant to salinity. Igbadun *et al.* (2006) showed that the crop yield response was very much dependent on the amount of water applied at different crop development stages than the overall seasonal water applied. Super absorbent polymer may have great potential in restoration and reclamation of soil and storing water available for plant growth and production. Incorporation of polymer into soil has increased wheat dry weight (Jounson and Leah, 1990). Super absorbent polymer can hold 400-1500 g of water per dry gram of hydrogel (Woodhouse and Johnson, 1991; Bowman and Evans, 1991). Due to the limited water resources in the world, it is essential to save and economize their use. All types of hydrogels when used correctly and in ideal situation will have at least 95% of their stored water available for plant absorption (Johnson and Veltkamp, 1985). The super absorbent polymer used under water deficit stress conditions, with improved moisture conditions, increased sink capacity, and provided a longer growing period (Tohidi Moghadam *et al.,* 2011). Many reports have been concerned with the impact of water scarcity ranging from several times to severe stresses in relation to interruptions in physiological processes in plants, variations in the metabolism of carbohydrates and nitrogen, modifications of protein structures and enzyme activity, the accumulation of proline, and the reduction of growth intensifier agents (Berar *et al.,* 1990; Levitt, 1980; Sing and Patal, 1996). This research aimed to identify variations occurring in soluble and insoluble carbohydrates in the leaves and roots of the safflower plant receiving supplemental irrigation at several growth stages.

Materials and methods

Study location

This experiment was conducted in western Iran during two farming years (2011-13) in a substation at the Lorestan Province Agricultural Studies and Natural Resources Research Center in the city of Khoramabad (longitude: 48°, 21´ E; latitude: 33°, 29´ N; altitude: 1171m). Mean precipitation rate is (440mm), mean annual temperature is 17.19°C, and annual evaporation rate is 1842.52mm (based on meteorological long-run statistics). The region has a semi-arid climate (according to Demarton and Anbrege's climatological coefficients). A control sample was prepared randomly and in zigzag form from 0-20 cm and 20-40 cm soil layers before cultivating the composed sample from farming soil, and its physical and chemical properties were measured (Table 2).

Treatments and agronomic operations

The current research was a split-plot factorial test with a fully randomized blocks design and three repetitions. The main factors were comprised of supplemental irrigation including the control sample without irrigation (T1), supplemental irrigation at late vegetative phase (I2) (175-180 days after cultivation), supplemental irrigation at flowering stage (I3) (195-200 days after cultivation), and supplemental irrigation at seed filling phase (I4) (210-215 days after cultivation). Two subsidiary factors included varieties (Padideh= V1, Goldasht = V2, Varamin 295 = V3, Zarghan 279, = V4, Mec.88 = V5, and Sina = V6), application of SAP at two levels of control group and lack of SAP (S1), and use of 200 kg per hectare SAP (S2). The varieties were well-known cultivars suitable for winter farming in the tested area, and their seeds were prepared by the Karaj Scion and Seed Preparation and Improvement Research Institute. The SAP (type: A200) was made by Rahab Resin Ltd. Co. under license from the Iranian Petrochemical and Polymer Institution. The maximum period of retention of this substance is 7 years in the soil, and the rate of its water absorption is about 220g/g in distilled water. SAP was put under seeds (15cm) simultaneously with stripped cultivation so that the plant's rhizomes could absorb more quickly the stored water in the SAP after water absorption and growth of buds. About 180g (30g in each 6m cultivation row) of SAP was consumed for each plot. Few experiments were conducted concerning the use of this substance in cultivating farm plants. The varieties prepared for study were sown inside trial plots on November 14th and 15th of 2011 and 2012, respectively. The dimensions of each experimental plot were 1.5m ×6m with 6 cultivation rows per plot. Cultivation rows were 0.25m wide, and a distance of 0.10m was between the bushes on the cultivation lines. A distance of 2m between major plots and 1m between sub-plots was maintained, and 5m was considered the interval between blocks. Nitrogen and phosphor fertilizers were consumed in amounts of 100kg per hectare pure nitrogen and 100kg per hectare P_2O_5 per year. Nitrogen fertilizer was applied at a rate of one-third at rosette stage, one-third at stemming phase, and one-third before late vegetative stage in all plots. Soil test results showed a sufficient amount of potassium; therefore no more fertilizer was needed. Winter wheat had been growing in the experimental site before the experiment. To compute the irrigation rate at given phases, the sample soil was prepared after measuring the rhizome depth; then water volume was calculated using the following formula:

$$V = \frac{(Fc - \Theta m) \times \rho a \times A \times ds}{Ea}$$

Where *V* denotes irrigation volume, *Fc* is humidity percentage within farm capacity, Ɵm is soil weight moisture percentage, *ρa* is soil bulk density, *A* is plot area, *ds* is approximate root penetration depth in cm at the given phase, and *Ea* is irrigation efficiency (it was considered to be 85%). According to Aforementioned equation, the volume of irrigation water was computed for each of developmental stages (Table 3).

Seed Yield

The grains of approximately 0.25 kg per plot were oven-dried to constant weight at 65°C and re-weighed to determine the water content. The grain yields were converted to a standard grain water content of 12%.

Soluble and Insoluble Carbohydrates measurement

To measure the studied physiological parameters, sampling was done a few days after the last irrigation at the seed filling phase (215-220 days after cultivation) in all plots. Relevant features of leaves were measured in green leaf samples taken from selected plants in each plot. Relevant root attributes were measured in samples extracted from a depth of 30cm. Sampling was performed in all experimental plots. soluble and insoluble carbohydrates rates were measured using Kochert's (1978) method.

Statics

Analyses were performed with using the MSTATC software. A split-factorial analysis of variance (ANOVA) was performed for all parameters. In addition the Duncan's Multiple Range Test (DMRT) ($P = 0.05$) was used to conduct mean comparison of treatments and find significant differences among means. Charts and figures were drawn with Microsoft office Word and Excel software, respectively.

Results and discussion

The findings of this study indicated that supplemental irrigation in various growth phases can affect a plant's internal products. Moreover, The various varieties showed various reactions to supplemental irrigation that was influenced by the growth duration and collision of growth stages with atmospheric conditions.

Table 1. Temperature, relative humidity and precipitation values measured during safflower crop cycle in 2011-12, 2012-13 and long-term data.

Periods	Metrological events	Months								
		November	December	January	February	March	April	May	June	July
Long-term	Temperature (°C)	13.9	8.4	5.6	6.0	9.8	13.4	18.5	24.6	28.8
	Relative humidity (%)	52.1	62.2	64.8	63.1	54.4	55.8	49.6	29.0	23.9
	Precipitation (mm)	57.5	73.7	55.6	66.4	50.8	90.8	33.5	1.8	0
2011-12	Temperature (°C)	8.1	4.8	3.8	5.6	8.7	15.4	22.5	28.5	30.8
	Relative humidity (%)	66	51	58.1	54.9	43.3	54.5	33.5	18.9	18
	Precipitation (mm)	65.9	0.6	11.4	53.1	13.4	100	11.5	0	0
2012-13	Temperature (°C)	11.9	6.4	4.7	7.7	12.2	15.9	19.7	27	30.2
	Relative humidity (%)	63.3	67.9	61.1	58	49.3	46.4	49.2	21.6	18.8
	Precipitation (mm)	34.0	77.3	73	29.9	12	33.1	68.4	0	0

Table 2. Soil chemical and some other important properties of the experimental soils.

years	Soil depth (cm)	Soil texture	pH	$CaCO_3$ (%)	EC ($ds.m^{-1}$)	N (%)	Cu (ppm)	Fe (ppm)	K (ppm)	P (ppm)	Organic matter
2011	0-20	Clay-loam	7.48	3.1	0.65	0.12	1	5.4	300	18	0.9
	20-40		7.63	3.8	0.71	0.14	0.08	4.5	264	14	0.6
2012	0-20	Clay-loam	7.39	3.4	0.62	0.11	1.1	5	338	15	1.0
	20-40		7.76	4.1	0.70	0.12	0.09	3.9	281	11	0.7

Soluble carbohydrate

Soluble carbohydrate levels in leaves were significantly affected by experimental treatments. Insoluble and reserved sacharides in the plant may be converted into soluble carbohydrates in order to increase the matric potential (Table 4). Supplementary irrigation can reduce the degradation of insoluble carbohydrates, thus decreasing the concentration of soluble carbohydrates in both leaves and roots (Table 5). In the second study year, the concentration of soluble carbohydrate was generally reduced in both leaves and roots compared with the first year. This was due to the additional precipitation in the second year. Also due to the occurrence of precipitation in the late vegetative stage of the second year, there was no significant difference between the concentrations of soluble carbohydrates in treatments I_1 and I_2. In both experimental years, soluble carbohydrate concentrations was higher in leaves than in roots (Fig 1). This fact might cause differences among the osmotic potential gradient, water flow, and nutrients from root to shoot and leaves. The researchers have mentioned a high correlation between the accumulation of soluble carbohydrates and rate of tolerance to drought stress in plants. Changes in carbohydrate, in addition to depending on severity and duration of water deficit, might also reflect genotypic differences in the regulation of carbon metabolism and partitioning at the whole plant level (Praxedes *et al.,* 2005). During the course of drought stresses active solute accumulation of compatible solutes such as carbohydrates is claimed to be an effective stress tolerance mechanism (McKersie and Leshem, 1994). Plants usually had the highest carbohydrate levels when grown under drought during both the vegetative phase and during anthesis. Owing to their solubility they may help plants to survive periods of osmotic stress induced by drought. The tolerant variety accumulated more soluble carbohydrate than the sensitive one.

Table 3. Irrigation water quantities applied to safflower at different stages of the experimental years.

Experimental years	Water application	Stage of development			Total stage	precipitation in Growth Season (mm)
		Late vegetative	Flowering	Yield formation		
2011-2012	Application day	178	201	208	246-250	
	Irrigation water (mm)	83	132	147		220
2012-2013	Application day	178	196	204	248-251	
	Irrigation water (mm)	64	118	136		320

Table 4. Pearson correlation coefficients among some of the characteristics that were measured in this study in two years.

	Leaf Soluble Carbohydrate	Root Soluble Carbohydrate	Leaf Insoluble Carbohydrate	Root Insoluble Carbohydrate
Seed Yield	-0.369*	-0.294	0.394*	0.540**
Leaf Soluble Carbohydrate		0.689**	-0.410**	-0.448**
Root Soluble Carbohydrate			-0.388*	-0.267
Leaf Insoluble Carbohydrate				0.595**

** and * : Correlation is Significant at the 0.01 and 0.05 Level, respectively.

Insoluble carbohydrates

The tested treatments significantly affected insoluble carbohydrates in both roots and leaves. The application of SAP during both testing years increased storage of saccharide substances and the concentration of insoluble carbohydrates in leaves and roots (Table 5). Implementing supplemental irrigation and removing the plant from stress conditions reduced the rate of degradation of insoluble carbohydrates and their conversion into soluble carbohydrates to create more negative osmotic potential. As a result, the rate of insoluble carbohydrates concentration increased. Since osmotic potential depends on the quantity of molecules in the soluble substance, osmosis function is regulated through the path of conversion of some insoluble carbohydrates like starch and fructane into soluble carbohydrates such as oligosaccharides, sucrose, and glucose (Hendry, 1993). Drought stress is deemed one of the paramount reducing factors for insoluble carbohydrates (Sabaghpour, 2004). The maximum storage of insoluble carbohydrate took place in leaves with supplemental irrigation at the late vegetative stage, and this indicates that supplemental irrigation in the late vegetative stage led to further storage of saccharide substances in leaves and more readiness in plants to enter the generative phase. The highest concentration of insoluble carbohydrates occurred in the roots with supplemental irrigation at the flowering stage (Table 5). Upon entering the generative phase and at the flowering stage, a plant's activities probably stop transiently. With supplemental irrigation at these stages and the absorption of nutrients by the roots, these substances are stored as insoluble carbohydrates in the roots, and with the reactivation of the plant, from the roots to the shoots flows.

Seed yield

Test results indicate that all treatments affected seed yield. Seed yield was noticeably increased with supplemental irrigation, and it was highly correlated with the amount of water received during the growth season. In the second year of testing, seed yield was improved by additional precipitation and its appropriate dispersion at all levels. In both the first year when precipitation was less and the second year when precipitation was more, supplemental irrigation increased seed yield at the flowering stage (Table 5). Thus, our findings indicate that the best time for supplemental irrigation is at flowering stage. reported that the highest damage on grain yield caused in drought stress at blooming (late vegetative stage) (Omidi, 2009 and Istanbulluoglu, 2009) and flowering stages in safflower (Omidi, 2009). Among the measured attributes, rhizome insoluble carbohydrate had the maximum, positively significant correlation with seed yield (Table 4). For this reason, the Sina cultivar produced a higher seed yield than other varieties. The application of SAP in all testing levels, by increasing the soil's water storage potential, caused significant increases in seed yield varying from 200-400kg per hectare. Previous studies revealed that if more SAP is mixed with the soil, the amount of moisture stored in the soil will also increase (Huttermannl *et al.,* 1999). FAO presents that good rain fed yields are in the range of 1.0–2.5 t ha_1; under irrigation in the range of 2.0– 4.0 t ha_1 from farmers' fields (Doorenbos and Kassam, 1979). Safflower yield data in different places under rain fed and irrigated conditions are also available for small experimental plots. For instance: in the Sacramento Valley of California, USA (Cavero *et al.,* 1999); in the Ariana of Tunisia (Hamrouni *et al.,* 2001); in the Pampas region of Argentina (Quiroga *et al.*, 2001); in the south of Italy (Lovelli *et al.*, 2007); in the Orissa of India (Kar *et al.,* 2007) in the range of 1.0–3.3 t ha_1. The yield obtained in this research was higher than the above presented values. Applied irrigation treatment is one of the most important reasons for this.

Table 5. Effects of supplemental irrigation (SI) and superabsorbent polymer (SP) treatments on yield, soluble and insoluble carbohydrates of Six safflower varieties (VA) in 2012 and 2013.

			Seed Yield kg.ha^{-1}		Soluble Sugar (mg.g^{-1} DW)				Insoluble Sugar (mg.g^{-1} DW)			
SI	VA	SP			Leaf		Root		Leaf		Root	
			2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
I1			1271c	1949c	41.01**a**	34.50**a**	24.02**a**	22.57**a**	14.90d	21.53b	9.583d	12.98b
I2			1827**a**	2218b	33.08b	33.32**a**	20.66b	20.75b	24.35**a**	23.81**a**	14.87b	15.63**a**
I3			1817**a**	2578**a**	31.55c	29.72b	19.14c	17.79c	21.79b	21.77b	16.16**a**	15.74**a**
I4			1437b	2540**a**	28.19d	28.08b	17.48d	16.01d	19.47c	20.58b	11.92c	13.25b
	G1		1595c	2207b	30.85d	29.80d	19.20d	17.21e	17.49d	22.40b	14.46b	13.90c
	G2		1727b	1839d	31.74cd	30.48cd	19.13d	18.36d	20.95b	24.29**a**	12.25d	15.92b
	G3		1300d	1895cd	34.90b	31.32bc	19.86cd	17.40de	20.16c	20.64c	11.25f	12.19e
	G4		1338d	2217b	32.15c	30.34cd	20.59bc	19.77c	19.83c	21.64b	13.04c	14.14c
	G5		1325d	2012c	36.94**a**	34.46**a**	21.91**a**	22.15**a**	19.47c	20.64c	11.88e	13.48d
	G6		2241**a**	3756**a**	34.18b	32.04b	21.26ab	20.77b	22.86**a**	21.93b	15.92**a**	16.76**a**
I1	G1		1265kl	1619ijk	36.60c	32.57cdef	24.76ab	20.93de	7.573**m**	22.73bc	10.31k	13.64jk
	G2		1395hijk	1571jk	44.30**a**	31.88defgh	22.83bcde	20.12ef	17.38ij	25.98**a**	8.715l	15.56cdef
	G3		1047m	1442**k**	40.50b	35.65ab	24.27abc	22.54bcd	15.66kl	15.50**f**	7.306**m**	7.837**o**
	G4		1206**l**	1853ghi	38.41c	35.06abc	22.49cde	22.07cde	16.44jk	22.13cd	10.05k	11.87m
	G5		1002m	1868ghi	40.94b	37.11**a**	25.46**a**	25.37**a**	14.74l	20.40de	9.086l	12.04m
	G6		1712f	3338c	45.33**a**	34.75abcd	24.32abc	24.37**ab**	17.60hij	22.48bcd	12.03ghi	16.91**a**
I2	G1		2055cd	2119fg	31.23ef	31.04efghi	18.51hijkl	17.72gh	25.36b	25.26**a**	17.56b	14.42hij
	G2		2181c	2006fgh	27.94ij	32.35cdefg	18.49hijkl	20.24ef	28.88**a**	26.04**a**	13.33f	15.86cde
	G3		1493gh	1853ghi	37.22c	32.58cdef	20.93defg	18.57fg	20.34ef	24.37**ab**	15.32d	15.98bcd
	G4		1333ijkl	1995fgh	33.35d	34.28abcd	20.75efgh	21.52cde	25.62b	22.79bc	14.12e	15.21defgh

	G5	1399hijk	1800hij	38.19c	36.47**a**	23.10bcd	23.57abc	19.90f	21.47cde	11.84hi	15.47defg
	G6	2501b	3535bc	30.55fgh	33.20bcde	22.19cdef	22.87bcd	25.99b	22.93bc	17.07bc	16.84**ab**
I3	G1	1660f	2624d	28.56hi	27.94jklm	16.94jklm	15.36ij	19.06fgh	21.20cde	17.36b	14.56ghi
	G2	1885e	1884ghi	27.66ij	29.10hijkl	19.23ghij	18.43fg	18.22ghi	22.91bc	15.92d	17.25**a**
	G3	1460ghi	2088fgh	33.17de	29.54ghijkl	17.96ijklm	15.70hij	23.07c	21.34cde	13.45f	14.85fgi
	G4	1500gh	2510d	30.76fg	26.80lm	20.10fghi	17.41ghi	19.83f	21.21cde	16.63c	16.45abc
	G5	1587fg	1981fgh	37.25c	34.40abcd	20.84defg	21.39cde	25.08b	21.24cde	14.30e	14.00ij
	G6	2808**a**	4382**a**	31.90def	30.57efghij	19.78ghi	18.43fg	25.51b	22.74bc	19.26**a**	17.31**a**
I4	G1	1400hijk	2465d	27.00ij	27.66jklm	16.62klm	14.85jk	17.98ghi	20.41de	12.60g	12.96kl
	G2	1448ghij	1896ghi	27.07ij	28.61ijkl	15.97**m**	14.67jk	19.31fg	22.22bcd	11.02j	15.02efgh
	G3	1200**l**	2199ef	28.71hi	27.52klm	16.30lm	12.80**k**	21.56de	21.34cde	8.931l	10.09n
	G4	1315jkl	2508d	26.07**j**	25.22**m**	19.03ghij	18.09fg	17.42ij	20.45de	11.35ij	13.03kl
	G5	1313jkl	2400de	31.36def	29.84fghijk	18.23ijklm	18.26fg	18.18ghi	19.47e	12.28gh	12.41lm
	G6	1946de	3770**b**	28.94ghi	29.64ghijkl	18.76ghijk	17.41ghi	22.36cd	19.59e	15.34d	15.99bcd
I1	S1	1140**g**	1667**e**	45.51**a**	38.54**a**	27.05**a**	24.72**a**	11.82**g**	19.79d	8.17**g**	11.32**d**
	S2	1402e	2231c	36.52b	30.47bc	21.00b	20.42c	17.97e	23.28b	10.99e	14.64b
I2	S1	1645d	1959d	36.42b	37.45 **a**	21.08b	23.38b	22.19c	21.96c	12.65d	14.29b
	S2	2009**a**	2477**b**	29.74d	29.19c	20.24b	18.11d	26.50**a**	25.66**a**	17.10b	16.97**a**
I3	S1	1732c	2294c	34.00c	31.91b	20.40b	20.21c	19.76d	19.44d	12.60d	14.66b
	S2	1901b	2862**a**	29.10d	27.54d	17.89c	15.36e	23.82b	24.10b	19.71**a**	16.81**a**
I4	S1	1280f	2164c	29.41d	30.41bc	18.25c	18.39d	16.20f	18.07**e**	9.490f	11.94c
	S2	1593d	2916**a**	26.98**e**	25.75**e**	16.72**d**	13.64**f**	22.74c	23.08b	14.35c	14.56b

Means within columns not followed by the same letter are significantly different at the p<0.05 level by Duncan's multiple range test.

Discussion and conclusion

The application of supplemental irrigation under dry-farming conditions, especially in regions where there is the possibility of this type of irrigation, could remove the stress conditions from the plant and improve seed yield. One of the important points in this method is the plant's stage of growth in which supplemental irrigation is implemented. In both years of the current study, given that the two testing years differed from each other in terms of rate and dispersion of precipitation, supplemental irrigation resulted in increased seed yield at the flowering stage more than other treatments. It was observed in this test that those substances which play determinant roles in the osmotic potential of plants always have a higher concentration in leaves than in roots, and this may cause the creation of an osmotic gradient to transfer water from the roots to the shoot. The role of variety (cultivar) in resistance against stress conditions is a function of a cultivar's characteristics in terms of physiological traits that influence growth and create seed yield. For example, in this experiment, one of the attributes affecting seed yield production was the rate of stored insoluble carbohydrates in the roots. Consuming SAP, especially when combined with the implementation of supplemental irrigation, provided more moisture for the plant and caused the given plant to be less exposed to stress conditions. Employing supplemental irrigation during the plants' critical need phases, applying SAP to increase the impact of supplemental irrigation, and using those plant varieties that have appropriate physiological characteristics for exposure to water shortage and stress conditions are valuable strategies for increasing seed yield under dry-farming conditions.

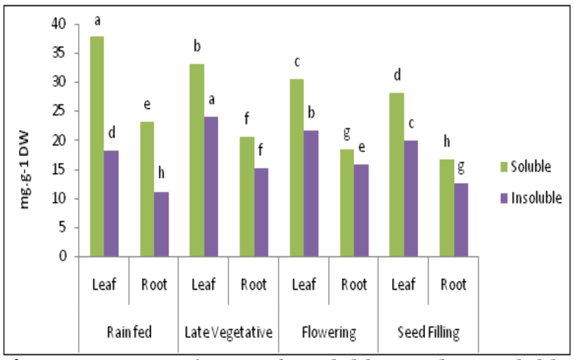

Fig. 1. Comparison of Soluble and Insoluble Carbohydrates between leaf and root affected by supplemental irrigation in both experimental years.

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