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Enhancing Drought Tolerance of Wheat (*Triticum aestivgum* L.) Through Foliar Application of Proline and L-Triptophan

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Abstract. Limited water availability is one of the important abiotic factor affecting yield of wheat crop. Exogenous application of osmolytes is an important factor in reducing the stress due to water shortage. Keeping in view the role of proline and L-tryptophan (L-TRP) in stress alleviation, a study was carried out at the agricultural research area of the University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan, during winter 2015-2016 to evaluate the impact of foliar applied proline and L-TRP on growth and photosynthetic efficiency of wheat grown under limited moisture supply. Drought stress was applied in three levels (I_1 = control, I_2 = drought stress at tillering stage and I_3 = drought stress at grain filling stage) while, foliar application of proline and L-TRP was done in six levels [T_1 = control; T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM)]. The experimental results revealed that growth and photosynthetic efficiency of wheat were decreased due to reduced water supply. However, exogenously applied proline and L-TRP considerably ameliorated the effect of drought stress. Combined application of L-TRP (10^{-4} M) and proline (20 mM) showed better results and induced tolerance to drought stress, in comparison with other treatments. Foliar application of proline and L-TRP also enhanced the photosynthetic rate which might be related with the improved photosynthetic pigments. Overall, exogenously applied proline and L-TRP mitigated the adverse effects of moisture deficit on growth and photosynthetic efficiency of wheat crop.

Keywords: wheat, drought, proline, L-tryptophan, foliar application

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop Worldwide. It is consumed as a staple food by one third of global population (Charkazi *et al*., 2010). Wheat is one of the most important energy sources for exorbitantly increasing population of the World (Debasis and Paramjit, 2003). Nonetheless, shortage of water, owing to climate variations, is an emerging issue in food production (Flexas *et al*., 2013). Among several abiotic factors which reduce the yield of wheat, drought is of prime importance. Scarcity of water is the major cause of variation in wheat yields in several regions of the World (Raza *et al*., 2012). A considerable part of agricul-tural land is severely affected by drought strain in the World which means drought stress is one of the most destructive ecological phenomena, depressing wheat yield Worldwide (Jamil *et al*., 2018). In Pakistan wheat is grown under extremely varying conditions of soil and climate. One third of the total area under wheat cultivation is rain-fed with uncommon rainfall (Khanzada *et al*., 2001). Limited water supply reduces leaf area, root development and stem elongation. Water utiliza-tion efficiency of the plant is also diminished due to disturbance in plant water relations (Farooq *et al*., 2009).

Osmolytes have been proved to serve as protective medium under stress and can be used very competently in crop plants for alleviating the effects of different

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kinds of abiotic stresses like drought (Bowne *et al*., 2012). They also concluded that proline activates several enzymes with a varied range of thermal sensitivities, and from different sources, against the *in vivo* inactivating effects of heat. A number of solutes (D-proline, hydroxyl proline, glycine, valine and glycerol), in addition to proline, demonstrate this stress ameliorating effect on plants. Hence it is concluded that the interaction between solute and enzyme protein is a general one with respect to both effecter and receptor molecules. The role of proline under drought stress includes osmotic adjustments, protection of membranes and enzyme system, scavenging of reactive oxygen species and conservation of energy and nitrogen to be utilized during exposure to abiotic stresses.

L-Tryptophan (L-TRP) is an essential unit for animals, plants and some bacteria (Meister, 1965). It is a precursors for a wide range of secondary metabolites (Less and Galili, 2008). It is considered as the main precursor for the synthesis of auxins in plant body, which plays a pivotal role in regulating the plant's growth and developmental processes (Wang *et al*., 2008). Several microorganisms release secondary metabolites upon the use of L-TRP. Being physiological precursor of auxins, L-tryptophan has more affirmative impact on growth and yield of plants than pure auxins (Zahir *et al*., 1999). A number of studies have reported the ameliorative role of L-TRP in improving physiological process of plants under different types of environmental stresses (Rao *et al*., 2012).

Materials and Methods

Site, experimental design and treatments. The current investigations were carried out during 2015-2016 at Agricultural Research Farm of the University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. It is situated on 29° N and 73° E. Bahawalpur is situated at the altitude of 159 m and has been characterized with very hot and cold climate during summer and winter, respectively. However, drought is a permanent feature of this region, throughout the year, except occasional showers. The maximum temperature rises to 48 °C while, minimum temperature falls to 7 °C. The trial was replicated thrice using randomized complete block design having split plot system. Drought stress treatments were allotted to main plots and foliar application of proline and L-tryptophan was allotted to sub plots. The net sub-plot size was 5m x 3m. Drought stress was given in three levels (I_1 = control; I_2 = drought stress at tillering stage and I_3 = drought stress at grain filling stage) while, foliar application of proline and L-TRP was done in six levels [T_1 = control; T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM)].

Data recording and analysis. Observations pertaining to growth and yield related traits were recorded according to standard procedures. Data on plant height was recorded by measuring randomly selected 5 plants in each subplot from base of plant to the tip of spikes including awns at physiological maturity. Fertile tillers (m^{-2}) were counted from an area of $1m^2$ at 3 randomly selected locations in each subplot at the time of harvest. Spike length (cm) was recorded by measuring randomly selected 5 spikes in each subplot. Number of spikelets per spike was recorded by counting the spikelets on randomly selected 5 spikes in each subplot. 1000 grains, collected from each subplot, were weighed using electronic balance to determine 1000 grain weight. Biological yield was calculated by weighing sundried plants per plot before threshing, followed by conversion in tons per hectare. Straw yield per plot was recorded and converted into tons per hectare. After threshing grain yield per plot was measured and converted into tons per hectare. Harvest index (%) was determined as described below:

$$\text{Harvest index (\%)} = \frac{\text{(Economic yield (grains)}}{\text{(Biological yield)}} \times 100$$

Chlorophylls 'a' and 'b' contents were analyzed as described by Lichtenthaler (1987). Fresh leaves (0.2 g) were excised and placed over-night with acetone (80%) at 4 °C, followed by centrifugation of the extract at 10,000 × g for 5 min. Spectrophotometer (Hitachi-U2001, Tokyo, Japan) was used to record the absorbance of the supernatant at 645, 663 and 480 nm. The chlorophylls 'a' and 'b' contents were determined as described below:

$$\text{Chlorophyll a } (\mu g/g) = [(12.7 \times \text{OD at } 663) - (2.69 \times \text{OD at } 645)]$$

$$\text{Chlorophyll b } (\mu g/g) = [(22.9 \times \text{OD at } 645) - (4.68 \times \text{OD at } 663)]$$

Flame photometer (Jenway, PFP-7) was employed to quantify the concentration of Na^+ and K^+ cations. Standard curves were obtained by using the standard solutions of Na^+ and K^+ (5 to 25 mg/L). The samples were run on flame photometer and the concentrations of Na^+ and K^+ were measured by comparing with the standard curves.

Leaf relative water content (RWC) was measured using the method described by Santos and Silva, (2015). Fresh weight of completely extended leaves was recorded immediately after their removal from the plants followed by soaking in distilled water for 4 h under a constant light at room temperature. The turgid weight of leaf was calculated and the samples were placed in oven for drying at 80 °C for 24 h. The dried samples were weighed and the relative water contents of the leaf were calculated by employing follow equation:

$$RWC = \frac{(\text{Fresh weight - Dry weight})}{(\text{Turged weight-Dry weight})} \times 100$$

Data collected was statistically analyzed by a computer program STATISTIX. Least significant difference test (LSD) at 0.05 probability level was employed to compare the difference among the treatment means (Steel *et al*., 1997).

Results and Discussion

Plant height, productive tillers and spike length. Data regarding plant height, productive tillers and spike length are shown in Table 1. Both treatments (reduced irrigation and spray of osmolytes) markedly affected plant height, number of productive tillers/m^2 and spike length. Highest value of plant height was observed in control irrigation (I_1) when sprayed with 20 mM proline (T_3), 10^{-4} M L-TRP (T_4), L-TRP + 10 mM proline (T_5) and L-TRP + 20 mM proline (T_6). At tillering and grain filling stages, the limited supply of irrigation considerably reduced the plant height. The plants exposed to drought stress at tillering stage exhibited the lowest value of plant height without the foliar application of osmolytes. The spray of osmolytes ameliorated the deleterious effect of drought stress at both stages. However, the application of L-TRP and Proline together proved to be more effective as compared with their individual treatment (Table 1). Data on productive tillers shows that the highest value was obtained in control irrigation treatment with foliar application of osmolytes. The maximum value of productive tillers was noted in drought stress at tillering stage when sprayed with

Table 1. Effect of drought stress and osmolytes on plant height, productive tillers and spike length of wheat (*Triticum aestivum* L.)

Drought level	Osmolyte treatment	Plant height	Productive tillers/m^2	Spike length(cm)
I_1	T_1	98.60ab	296.67a-c	11.67a-c
	T_2	99.03ab	302.27ab	11.87a-c
	T_3	99.80a	306.83ab	12.17ab
	T_4	99.77a	309.67ab	12.30ab
	T_5	101.83a	310.47a	12.57ab
	T_6	103.00a	319.13a	12.73a
I_2	T_1	80.33f	280.50a-c	10.10c
	T_2	81.63f	282.90a-c	10.33bc
	T_3	87.00e	285.23a-c	10.43bc
	T_4	89.63c-e	272.57c	10.80bc
	T_5	91.37c-e	275.83bc	10.97bc
	T_6	93.23c	275.83bc	11.50a-c
I_3	T_1	88.23de	276.43bc	10.30bc
	T_2	87.13e	281.03a-c	10.50bc
	T_3	89.67c-e	281.73a-c	10.73bc
	T_4	89.97c-e	283.43a-c	11.03a-c
	T_5	92.73cd	289.97a-c	11.13a-c
	T_6	94.23bc	293.70a-c	11.43a-c

Means sharing the common letters are statistically non-significant at 5% probability level (n=3). I_1 = control (normal irrigation); I_2 = drought stress at tillering stage; I_3 = drought stress at grain filling stage; T_1 = control (distill water spray); T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM).

proline 20 μm. However, combined treatment of proline and L-TRP mitigated the impact of drought and enhanced the tillering capacity of the plants, as compared with the control. The data concerning spike length revealed that drought stress caused a significant decline in the value. However, individual as well as combined application of osmolytes caused a significant enhancement in spike length (Table 1).

Photosynthetic pigments. Data regarding chlorophyll a and b contents are shown in Table 2. Both treatments; foliar application of osmolytes and irrigation levels significantly affected chlorophyll a and b contents. Drought stress at both stages (tillering and grain filling) significantly reduced chlorophyll a and b contents. However, foliar application of proline and L-TRP mitigated the impact of reduced moisture supply and increased the contents of photosynthetic pigments (Table 2).

Sodium, potassium and relative water contents. Data regarding Na and K reveals that drought stress increased Na^+ and decreased K^+ contents. The values of Na^+ and K^+ were significantly decreased and increased, respectively, by foliar application of osmolytes individually as well as in combination (Table 3). Both treatments; foliar application of osmolytes and limited moisture supply, revealed a significant effect on Na^+ and K^+ contents in leaves. T_4I_3 gave the highest value (49.48 ppm) of Na^+ contents in leaves and T_5I_3 gave the highest value (17.67 ppm) of K^+ contents in leaves. Minimum value (45.67 ppm) of Na^+ contents in leaves was observed in T_1I_1 which was 7.43% less than the highest value and the minimum value (15.00 ppm) of K^+ content in leaves was observed in T_3I_1 which was 15.09% less than the highest one.

Data regarding relative water contents in leaves (%) is shown in the Table 3. Both treatments; foliar application

Table 2. Effect of drought stress and osmolytes on photosynthetic pigments of wheat (*Triticum aestivum* L.)

Drought level	Osmolyte treatment	chlorophyll (a) contents (μg/g fresh weight)	chlorophyll (b) contents (μg/g fresh weight)
I_1	T_1	0.963g	0.04a
	T_2	1.07fg	0.03ab
	T_3	1.50a	0.01ab
	T_4	1.07fg	0.04a
	T_5	1.10e-g	0.03ab
	T_6	1.32b-d	0.03ab
I_2	T_1	1.28c-e	0.02ab
	T_2	1.47ab	0.01ab
	T_3	1.45a-c	0.003b
	T_4	1.56a	0.008ab
	T_5	1.44a-c	0.01ab
	T_6	1.05fg	0.02ab
I_3	T_1	0.969g	0.04a
	T_2	1.22d-f	0.02ab
	T_3	1.40a-c	0.02ab
	T_4	1.01g	0.03ab
	T_5	1.12e-g	0.02ab
	T_6	1.00g	0.03ab

Means sharing the common letters are statistically non-significant at 5% probability level (n=3). I_1 = control (normal irrigation); I_2 = drought stress at tillering stage; I_3 = drought stress at grain filling stage; T_1 = control (distill water spray); T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM).

Table 3. Effect of drought stress and osmolytes on sodium, potassium and relative water contents of wheat (*Triticum aestivum* L.)

Drought level	Osmolyte treatment (ppm)	Sodium content (ppm)	Potassium content (%)	Relative water content in leaves
I_1	T_1	45.67b	16.33ab	63.13a-d
	T_2	46.43ab	16.67ab	74.63a-c
	T_3	46.67ab	15.00b	70.83a-c
	T_4	46.67ab	16.33ab	75.97a-c
	T_5	49.28a	15.67ab	79.17a-c
	T_6	48.67ab	15.33ab	84.13ab
I_2	T_1	49.17a	15.33ab	44.60c
	T_2	48.51ab	16.33ab	50.93cd
	T_3	48.29ab	16.00ab	55.93b-d
	T_4	49.39a	16.00ab	55.80b-d
	T_5	49.34a	15.33ab	62.80a-d
	T_6	47.39ab	17.00ab	66.83a-d
I_3	T_1	49.32a	15.00b	40.13d
	T_2	48.41ab	16.67ab	57.20b-d
	T_3	46.53ab	16.00ab	61.00a-d
	T_4	49.48a	16.33ab	61.67a-d
	T_5	48.33ab	17.67a	63.10a-d
	T_6	48.09ab	17.67a	69.87a-d

Means sharing the common letters are statistically non-significant at 5% probability level (n=3). I_1 = control (normal irrigation); I_2 = drought stress at tillering stage; I_3 = drought stress at grain filling stage; T_1 = control (distill water spray); T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM).

of osmolytes and irrigation levels showed significant effect on RWC in leaves. Among the interactions, T_6I_1 produced the highest value (84.13%) of leaf RWC. Nonetheless, the minimum value (40.13) leaf RWC was observed in T_1I_3 which was 52.35% less than the highest one. Individual as well as combined application of osmolytes considerably improved the relative water content of leaves under reduced irrigation.

Biological yield of seed and straw, harvest index and 1000 grain weight. Data on yield attributes are presented in Table 4. Biological yield was severely declined by drought stress at both growth stages under study (tillering and grain filling). Minimum value of biological yield was recorded in those plots where drought stress was imposed at grain filling stage followed by drought stress at tillering stage, without the application of osmolytes. However, foliar application of osmolytes individually, as well as in combination ameliorated the stress and significantly enhanced the biological yield. Maximum biological yield was noted under normal irrigation when sprayed with both osmolytes (L-TRP + 20 mM proline) in T_6I_1.

Grain yield also exhibited the similar behaviour. Minimum grain yield was recorded under drought stress at grain filling stage followed by tillering, without foliar application of osmolytes. Individual as well as combined treatment of plants with proline and L-TRP, mitigated the drought stress and caused a considerable enhancement in grain yield. Maximum grain yield was recorded in normal irrigation under combined application of both osmolytes (L-TRP + 10 mM proline). Straw yield showed the similar behavior as that of biological yield and grain yield.

Harvest index was severely reduced by drought stress at tillering as well as grain filling stage. The highest reduction in harvest index was noted in those plants where drought stress was imposed at grain filling stage without the spray of osmolytes, while lowest reduction was observed under normal irrigation with combined application of L-TRP and 20 mM proline T_6I_1. Nonetheless, application of osmolytes under drought stress at both stages (Tillering and grain filling) improved the harvest index.

Table 4. Effect of drought stress and osmolytes on biological yield, grain yield, straw yield, harvest index and 1000 grain weight of wheat (*Triticum aestivum* L.)

Drought level	Osmolyte treatment	Biological yield (t/ha)	Grain yield (t/ha)	Straw yield (t/ha)	Harvest index (%)	1000 grain weight (g)
I_1	T_1	10.50a-c	4.33ac	6.47a-c	44.1a-c	48.33b-d
	T_2	10.30a-c	4.90ac	6.37a-c	47.89a-c	46.00c-e
	T_3	10.43a-c	5.03ac	6.13a-c	48.26ab	51.00a-c
	T_4	11.07ab	5.16ab	6.17a-c	46.69a-c	51.33a-c
	T_5	11.77ab	5.36a	6.83ab	45.65a-c	53.33ab
	T_6	13.13a	4.6a-c	7.97a	51.29a	56.33a
I_2	T_1	8.07b-d	3.13bc	4.03d	42.44a-c	41.00ef
	T_2	8.53a-d	3.23bc	4.70b-d	38.40a-c	41.33ef
	T_3	8.03cd	3.37bc	4.67a-d	42.26a-c	43.00def
	T_4	8.93a-d	3.37bc	5.13a-d	35.51bc	46.00c-e
	T_5	9.50a-d	3.92bc	5.28a-d	41.56a-c	47.00c-e
	T_6	9.87 a-d	4.02a-c	5.85a-d	41.07a-c	48.33b-d
I_3	T_1	6.57d	2.80c	4.10cd	33.47c	38.67f
	T_2	8.27a-d	3.27bc	5.00ad	39.22a-c	44.67d-f
	T_3	8.00cd	3.30bc	4.50b-d	42.49a-c	42.67d-f
	T_4	8.57a-d	3.27bc	5.13a-d	34.95bc	43.33d-f
	T_5	9.50a-d	3.90bc	5.60a-d	41.13a-c	47.33c-e
	T_6	10.00a-c	4.10a-c	5.90a-d	40.99a-c	49.00b-d

Means sharing the common letters are statistically non-significant at 5% probability level (n=3). I_1 = control (normal irrigation); I_2 = drought stress at tillering stage; I_3 = drought stress at grain filling stage; T_1 = control (distill water spray); T_2 = proline (10 mM); T_3 = proline (20 mM); T_4 = L-TRP (10^{-4} M); T_5 = L-TRP (10^{-4} M) + proline (10 mM) and T_6 = L-TRP (10^{-4} M) + proline (20 mM).

Drought stress at both developmental stages caused a pronounced decline in 1000-grain weight. Maximum reduction in 100-grain weight was induced by drought stress at grain filling stage. However, application of osmolytes significantly improved the value under drought stress at both growth stages (tillering and grain filling).

Plant height is an important growth regulating parameter. In present study, drought stress negatively affected the plant height because water stress was given to the crop at tillering and grain filling stage, but foliar application of osmoprotectants significantly affected the plant height. Among the interactions, T_6I_1 [NPK + L-tryptophan + proline (20 mM) with controlled irrigation] gave the highest value of plant height (103.00 cm). Similar observations have also been reported by previous researchers (Raza *et al*., 2012; Ashraf and Foolad, 2007).

Number of productive tillers per m^2 is directly related to the final yield. Interaction of foliar application of osmoprotectants and drought levels showed significant effect on number of productive tillers per m^2. Among the interactions, T_6I_1 [NPK + L-tryptophan + proline (20 mM) with controlled irrigation] depicted the highest number of productive tillers per m^2 (319.13). These results are also corroborated with previous findings (Raza *et al*., 2012; Ashraf and Foolad 2007).

Spike length is an important yield attributing trait. Yield of wheat crop is directly related to spike length as larger spikes contain more number of spikelets and ultimately the yield increases. Interaction of foliar application of osmolytes and drought levels showed significant effect on spike length (cm). Among the interactions, T_6I_1 [NPK + L-tryptophan + proline P_2 (20 mM) with controlled irrigation] showed the highest spike length (12.73 cm). Similar results were found by previous scientists (Irfan *et al*., 2006; Abdel-Hameed *et al*., 2004) who reported that foliar application of osmolytes has favourable effects on growth and yield of plants.

1000-grain weight is one of the most important components of wheat crop signifying the growth and development of grains, which ultimately contributes towards the final yield. Foliar application of osmolytes significantly affected the 1000-grain weight (g). Execution of T_6 [NPK + L-tryptophan + proline P_2 (20 mM)] treatment revealed the maximum 1000-grain weight (51.22 g). Similar findings were observed by Talaat and Youssef (2002).

Biological yield signifies the total biomass produced by the plant during its lifecycle by consumption of available inputs. Interaction of drought levels and foliar application of osmoprotectants showed significant effect on biological yield. Among the treatments, T_6I_1 [NPK + L-tryptophan + proline P_2 (20 mM) with controlled irrigation] gave the highest biological yield (13.13 t/ha). The results of the present study are in line with Ashraf and Foolad (2007).

Grain yield is the final product of the interaction of all the contributing parameters like spike length, number of spikelets per spike and finally grain weight. Any fluctuation in these components directly affects the final yield. Exogenous application of proline and L-tryptophan under drought stress increased the grain yield by reducing the harmful effects of drought. T_5I_1 [NPK + L-tryptophan + proline P_1 (10 mM) with controlled irrigation] produced the highest grain yield (5.36 t/ha). These results are in line with the findings of previous investigations (Irfan *et al*., 2006; Abdel-Hameed *et al*., 2004).

Potassium is an important factor for osmoregulation, stimulates enzymes of respiration and photosynthesis and plays a significant role in stomatal regulation. Concentration of potassium in leaves of wheat was decreased significantly due to reduced water supply at various growth stages. Exogenously applied proline and L-tryptophan at all growth stages significantly increased the shoot K^+ under stress conditions in wheat crop. However, this increase was more obvious when combination of L-tryptophan proline and was applied @ 10^{-4} M and 10 mM, respectively. T_5I_3 [NPK + L-tryptophan + proline P_1 (10 mM) with water stress at grain filling stage] illustrated the highest value (17.67 ppm) of K^+ contents in leaves. Similar results have also been revealed during previous studies (Abdel-Hameed *et al*. 2004).

According to previous studies, chlorophyll 'a' and 'b' contents are extremely important in photochemical reactions (Santos and Silva, 2015). Water-deficit stress significantly reduced the chlorophyll 'a' and 'b' contents in present investigation. However, foliar application of osmolytes (proline and L-tryptophan) showed significant improvement in photosynthetic pigment contents. Similar findings were also observed by Ali *et al*. (2007) and Kaya *et al*. (2006).

Water-deficit stress negatively affected the relative water contents. The reduction in this attribute may be due to deficiency of chlorophyll and other photosynthetic pigments (Santos and Silva, 2015). Water deficit affected the leaf expansion and decreased the total surface area of leaves due to which water status of plants and relative water contents of leaf were affected. Foliar application of osmoprotectants under scarce moisture supply enhanced relative water contents in leaves. T_6I_1 [NPK + proline P_2 (20 mM) + L-tryptophan 10^{-4} M with controlled irrigation] gave the highest value (84.13%) of relative water contents in leaves. These results are in line with those reported by Kafi (2009).

Conflict of Interest. The authors declare no conflict of interest.

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