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Water Deficit Effects on Proline, Cell Wall Elasticity and Osmotic Potential among seven *Avena* species at Vegetative and Flowering stages

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ABSTRACT: This paper has concentrated to quantify the effect of water limitation in *Avena* species at different phenophases in terms of plant water relations and osmotic adjustment responses triggered by drought stress. Relative Water content (RWC) decreased in all the species when the drought period progressed in subsequent days after with-holding water supply. At vegetative stage all the seven species of *Avena* grouped under three categories in response to the extent of moisture stress. The group one comprised of three species viz. *A. brevis, A. maroccana* and *A. sterilis* showing the maximum stress up to 16th day from with-holding the water supply. The second group comprised of three species (*A. strigosa*, *A. vaviloviana* and *A. abyssinica*) showing tolerance up to 12th day and the third group in which the stress tolerance was recorded up to 9th day comprised of only one species i.e. *A. sativa*. Among all these seven species evaluated for drought tolerance, three species belonging to group Ist showed better water status. *A. sterilis* was found to be the relatively more tolerant species among the three in terms of highest percentage of proline accumulation with a moderate level of osmolality concentration and RWC percentage. In the 2nd group, *A. strigosa* showed maximum toleranance up to 12th day with minimum decrease in water and osmotic potential, higher accumulation of osmolality, less decrease in RWC and moderate accumulation of proline. *Avena sativa*, the most abundant cultivated species of *Avena* showed the degree of tolerance up to 9th day only. Hence, *A. sterilis* can be considered as the highest tolerant species under water deficit conditions at vegetative stage followed by *A. strigosa* and *A. sativa*. When water stress was imposed at flowering stage, six species of *Avena* (*A. strigosa, A. brevis, A. vaviloviana, A. abyssinica, A. maroccana* and *A. sativa*) showed tolerance up to 5th day only and one species *A. sterilis* up to 4th day. Among the six species, *A. maroccana* showed better moisture retention capacity in terms of less decrease in RWC percent with a moderate increase in osmolality, proline, less decrease in water potential, osmotic potential followed by *A. abyssinica*.

Keywords: Drought, water relations, tolerance, climate change, oat, stress.

INTRODUCTION

Oat is ranked sixth in world cereal output, after wheat, maize, rice, barley, and sorghum (Ivanov, 2006), and seventh in world cereal cultivated area (FAO, 2013). It thrives in poor and unfavourable environments and is mostly used as a fodder crop across the world (Loskutov and Rines, 2011). It includes roughly 10%–12% protein and 30–35% dry matter, making it a balanced diet for cattle, sheep, and other domestic animals. Grains are commonly utilised as cow fodder. Oatmeal is a common breakfast ingredient in most industrialised nations since it is a good source of -gluten proteins and has a low prolamine concentration (Gorash *et al*., 2017). Because oats are a good source of antioxidants, they may help with the treatment of type 2 diabetes by stabilising blood sugar levels (Zhang *et al*., 2014; Hou *et al*., 2015) and cancer (Boffetta *et al*., 2014). Oats are the most significant grain fodder crop in India, and it is cultivated in the winter season in the North Western and Central regions, as well as the eastern area. Oat is produced in the plains and hilly sections of the nation since it demands a lengthy and cold growing season. Punjab, Haryana, Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Rajasthan, Maharashtra, and West Bengal are among the Indian states where it is grown. Oats are a high-producing crop, yielding 45-55 tonnes of green feed per hectare on average. Oats may be cultivated in a wide range of soil types.

The genus Avena belongs to the Poaceae family and has roughly 31 species, both wild and cultivated, that have been classified based on genome, ploidy level, and range (Loskutov and Rines 2011). The primary oats farmed for fodder and grain purposes are *Avena sativa* and *Avena byzantina*. Water availability is generally the key factor impacting agricultural yield in dry locations in the era of climate change and ever-increasing global population, hence methods aimed at enhancing sustainable water use and plant drought tolerance are urgently needed (Erice *et al*., 2010). Drought stress is one of the leading causes of crop loss throughout the world, with average yields dropping by 50% or more (Wang *et al.*, 2003). Water stress is described as the induction of turgor pressure below the maximum potential pressure, which causes a water deficit in plant tissue and, as a result, a major reduction in photosynthesis. Drought tolerance hinges on the capacity to retain photosynthetic machinery performance under water stress. In most dry and semi-arid environments, water scarcity is the most significant barrier to fodder production and yield stability (Shao *et al*., 2009).

The relative water content (RWC) of a leaf has long been used to determine its water condition. RWC, which may be used as an integrated metric of plant water status, can be used to assess metabolic activity in leaf tissue. Drought resistance in cereals has been demonstrated to be a quantitative trait, and RWC is a reliable technique for assessing drought tolerance (Teulate *et al*., 2003). Cereals' physiological and morphological responses to water stress were investigated (Blum, 2005). Water scarcity inhibits plant growth and agricultural yield more than any other environmental element in arid and semi-arid countries. Plants have developed a variety of defensive systems that enable them to live and flourish in harsh settings, as well as adapt to water deficiency stress through a variety of physiological and molecular pathways (Anjum *et al*., 2011). Crop When levels of resistance to various biotic and abiotic stresses in cultivated germplasm are low or the range of genetic variability is narrow, selection pressure results in virulent biotypes of pests and diseases, finding and incorporating additional genes for resistance from wild species becomes critical to maintaining crop productivity (Upadhyay *et al*., 2014). The problem of environmental pressures may be solved through genetic modification of plants by breeding and identification of germplasm for their growth and yield under adverse circumstances. Understanding tolerance mechanisms at the morphological, physiological, and biochemical levels leads to the identification of genotypes/species that can adapt to water deficit and maintain growth, development, and productivity during stressful periods, particularly in arid and semi-arid environments.

In the present experiment, seven *Avena* species comprising *Avena sativa* and *Avena sterilis* (*weedy A. fatua*) belonging to primary gene pool; *A. maraccona* from secondary genepool and *A. abyssinica, A. vaviloviana*, *A. strigosa* and *A. brevis* from tertiary gene pool having different degrees of resistance to different diseases were selected. *Avena sterilis* (*weedy A. fatua*) is the progenitor of cultivated oats and serves as a multiple resistance source to several oat diseases viz. crown rust, stem rust, powdery mildew, *Fusarium*, head blight, leaf blotch, smut, cereal cyst nematode resistance and barley yellow dwarf virus (BYDV). It is commonly used in several oat breeding programmes across the world (Frey 1985). *A. maroccana* for crown rust disease, *A. vaviloviana* for powdery mildew, crown rust, BYDV, smut; *A. abyssinica* for powdery mildew, crown rust, stem rust and smut and *A. strigosa* for crown rust, stem rust and smut. The present work aims at studying the plant water relations and associated osmolyte accumulation in seven *Avena* species grown under water limited environment to identify drought tolerant species for utilization as donor in oat breeding programme.

MATERIALS AND METHODS

Avena strigosa, *Avena brevis, Avena vaviloviana, Avena abyssinica, Avena sativa, Avena maroccana*, and *Avena sterilis* seeds were sowed in porcelain pots (size 30×20cm) filled with 20 kg garden soil and a 2:1 ratio of farmyard manure. Three seedlings were kept in each container after uniform germination. Water stress was generated by withholding watering during the vegetative and blooming stages until the plant reached the permanent wilting point (PWP). A second batch of each species was kept irrigated to 100 percent of the field capacity.

Relative water content (RWC): The relative water content (RWC) was calculated using Barrs and Weatherley's approach (1962).

Leaf water potential (WP): The leaf water potential was calculated with a thermocouple psychrometer and Wescor C-52 chambers linked to an HR33T Dew point microvolt metre. The recorded result was then divided by the proportionality constant (-0.75 moles/bar) to obtain the water potential in bar, which was then translated to Mega Pascal (MPa).

Osmolality and Osmotic Potential: The osmolality of the cell sap was determined using a vapour pressure osmometer (5500, Wescor, Inc USA). The osmolality (mmoles/kg) was calculated using the results. These values were further converted into pressure units (MPa) using the following equation.

$$\text{OP (MPa)} = -R \times T \times \text{moles /kg (osmolality)}$$

The obtained OP was further corrected for the dilution of symplastic sap by apoplastic water by using equation (OP+0.1 OP). The osmotic potential obtained at full turgor denoted as OP100 was calculated according to Wilson et al. (1980) using the following equation.

$$\text{OP100} = (\text{corrected OP} \times \text{RWC})/100$$

Estimation of Proline: Proline content was estimated spectro-photometrically in the leaves of both control and stressed plants at both vegetative and flowering stage according to method of Bates *et al.,* (1973). It was expressed as µmoles g^{-1} FW.

RESULTS AND DISCUSSION

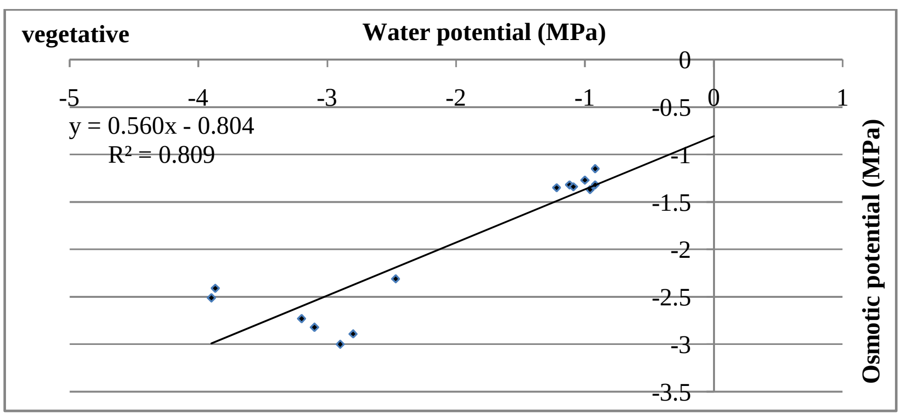
In the present study, seven species of oat were evaluated to study the effect of water deficit on RWC, osmotic potential, leaf water potential and proline accumulation. Relative water content and water potential are indices of plant water status, which are useful in monitoring the development of stress in plants which are growing under drought conditions. Maintenance of turgor by osmotic adjustment provides a major physiological means for minimizing the detrimental effect of drought stress in some species. Three species *A. brevis, A. maroccana* and *A. sterilis* were comprised in group 1 based on observations recorded when stress was imposed at vegetative stage (Table 1).

Table 1: Performance of seven *Avena* species responding to different periods of water stress at vegetative stage.

Species	Vegetative Stage									
	Relative water content (%)		Water Potential (MPa)		Proline (μ mole g^{-1} fw)		Osmotic Potential (MPa)		Osmolality (m mol kg^{-1})	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Maximum stress achieved on 16th day after withholding water										
A. brevis	86.02	50.84	-1.20	-3.80	18.0	526.5	-1.34	-2.41	542.5	972.5
A. maroccana	84.10	48.80	-0.90	-2.8	15.4	613.0	-1.17	-2.89	471.5	1167.0
A. sterilis	86.96	47.91	-1.13	-3.90	12.6	648.0	-1.37	-2.51	553.5	1015.0
Maximum stress achieved on 12th day after withholding water										
A. strigosa	84.74	54.29	-1.11	-2.90	17.4	316.5	-1.41	-3.01	571.0	1213.0
A.vaviloviana	85.21	42.86	-1.23	-3.10	30.3	875.5	-1.35	-2.82	544.5	1138.0
A. abyssinica	85.32	42.41	-0.93	-2.47	23.7	818.0	-1.33	-2.31	537.5	934.0
Maximum stress achieved on 9th day after withholding water										
A. sativa	87.21	43.65	-1.27	-3.67	12.4	596.0	-1.41	-3.22	569.0	1298.0

Table 2: Performance of seven Avena species responding to different periods of water stress at flowering stage.

Species	Flowering Stage									
	Relative water content (%)		Water Potential (MPa)		Proline (μ mole g^{-1} fw)		Osmotic Potential (MPa)		Osmolality (m mol kg^{-1})	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Maximum stress achieved on 5th day after withholding water										
A. strigosa	82.99	33.4	-1.25	-7.47	52.45	990.50	-1.27	-3.22	512.8	1299.5
A. brevis	78.86	26.34	-1.81	-5.6	14.02	580.00	-1.26	-2.78	510.2	1121.0
A.vaviloviana	79.78	31.84	-1.54	-4.43	17.35	516.00	-1.17	-2.71	470.4	1095.5
A. abyssinica	78.45	47.76	-1.40	-6.27	13.57	425.50	-1.35	-2.47	546.0	997.0
A. maroccana	69.93	47.40	-2.72	-3.97	16.44	129.65	-1.83	-2.46	736.9	994.0
A. sativa	83.92	40.44	-1.97	-4.57	12.31	640.50	-1.56	-1.74	628.2	1183.5
Maximum stress achieved on 4th day after withholding water										
A. sterilis	80.72	49.07	-2.092	-3.967	18.08	165.15	-2.01	-3.25	811.9	1313.0



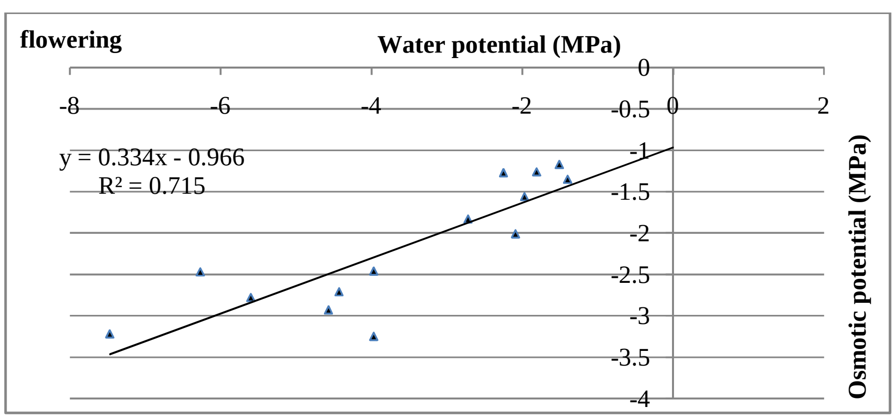


Fig. 1. Correlation coefficient of osmotic potential and water potential both at vegetative and flowering stage.

Among these *A. brevis* showed 50.84% RWC with a water potential and Osmotic potential value of -3.87 MPa and -2.41 MPa respectively. The osmolality ranged from 542.50 m molekg^{-1} (1^{st} day) to 972.50 mmole kg^{-1} (16^{th} day). At flowering stage, the maximum stress occurred on the 5^{th} day may be due to the higher transpiration owing to higher biomass accumulation at flowering stages. The RWC decreased from 78.862% on the 1^{st} day to 26.348% on the 5^{th} day, with a water potential value of -5.6 MPa, osmotic potential -2.777 MPa, osmolality of 1121 mmoles kg^{-1}. The proline content increased from 14.020 µmole g^{-1} fw to 580.00 µmole g^{-1} fw and it is shown in Table 2 and Fig. 3. *A. maroccana* showed a decrease in RWC from 84.10% on the 1st day to 48.80% on the 16^{th} day of stress, with the decrease in water potential of -0.90 MPa on the 1^{st} day to -2.80 MPa on 16^{th} day. The osmotic potential and osmolality varied from -1.17 MPa to -2.89 MPa and 471.50 mmolekg^{-1} to 1167.00 mmole kg^{-1} respectively. Proline showed an increase from 15.40 µmole g^{-1} fw on the 1^{st} day to 613 µmole g^{-1} fw on the 16^{th} day. At flowering stage, the RWC decreased from 69.93% to 47.40% on the 5^{th} day stress of the plant with a Water potential value of -3.967 MPa, Osmotic potential of -2.462 MPa, osmolality of 994.00 mmoleskg^{-1}, Proline of 129.650 (Table 2). *A. sterilis* showed 47.91% RWC with a water potential and osmotic potential value of -3.90 MPa and -2.51 MPa respectively on the 16^{th} day. The osmolality varies from 553.30 mmolekg^{-1} on the 1^{st} day to 1015 mmolekg^{-1} on the 16th day, with an accumulated proline to 648.00 µmole g^{-1} fw. At flowering stage RWC goes from 80.717% on the 1st day to 49.065% on the 4th day where maximum stress symptom occur (Table 2).

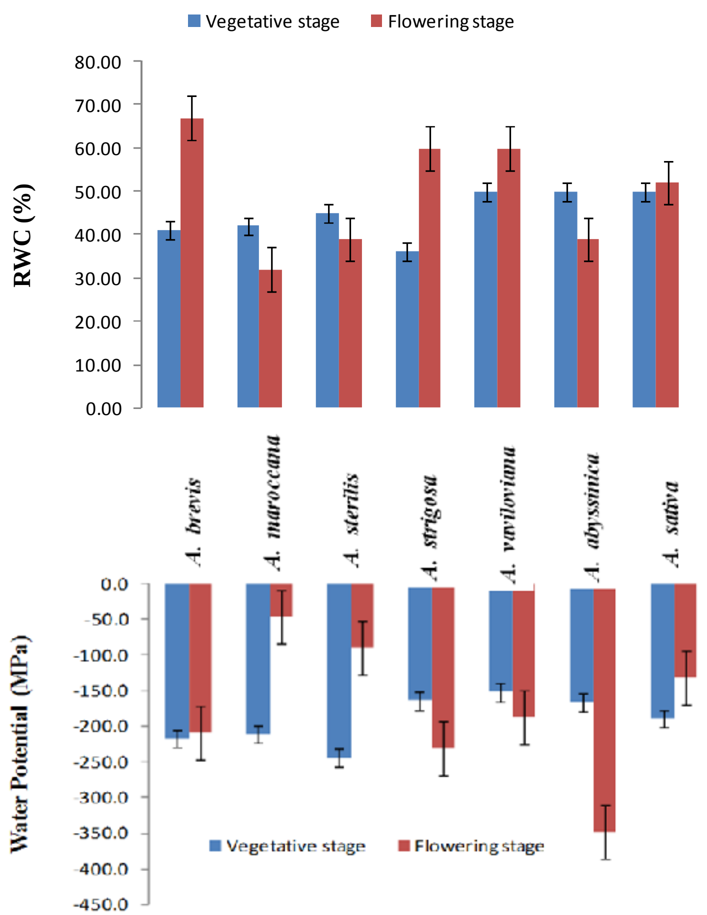


Fig. 2. Comparison of percent increase in Relative water content and Water potential over control in different Avena species under moisture stress at vegetative and flowering stages.

Among group 2, which again comprised of three species (*A. strigosa, A. vaviloviana* and *A. abyssinica*) achieved maximum stress condition at 12^{th} day of water withholding at vegetative stage and at 5^{th} day of water withholding at flowering stage. At maximum stress, the RWC value of A. *strigosa* was reduced from 84.74 % to 54.29% with water potential of -2.90 MPa, osmotic potential of -2.16 MPa, osmolality of 1213 m mole kg^{-1} and proline content of 316.5 µmole g^{-1} fw at vegetative stage. Similarly the value was found to be 33.405%, 990.5 µmole g^{-1} fw, -4.433 MPa, -2.714 MPa and 1095.5 m mole kg^{-1} respectively for the same parameters at flowering stage (Table 2). In *A. vaviloviana*, RWC reduced from 85.21 to 42.86% with water potential of -3.10 MPa, osmotic potential of -2.81 MPa, osmolality of 1138 m mole kg^{-1}, proline of 875 µmole g^{-1} fw, at vegetative stage (Table 1) and at flowering stage the same parameters showed a value of 31.853%, 516 µmole g^{-1} fw, -4.433 MPa, -2.714 MPa and 1095.5 m mole kg^{-1} respectively (Table 2).

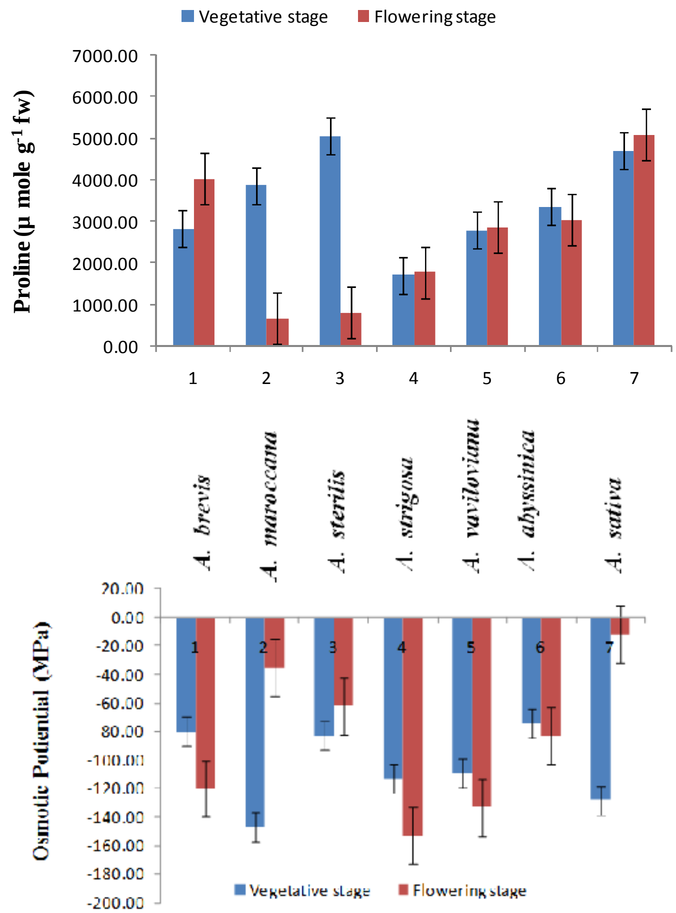


Fig. 3. Comparison of percent increase in Proline and Osmotic potential over control in different *Avena* species under moisture stress at vegetative and flowering stages.

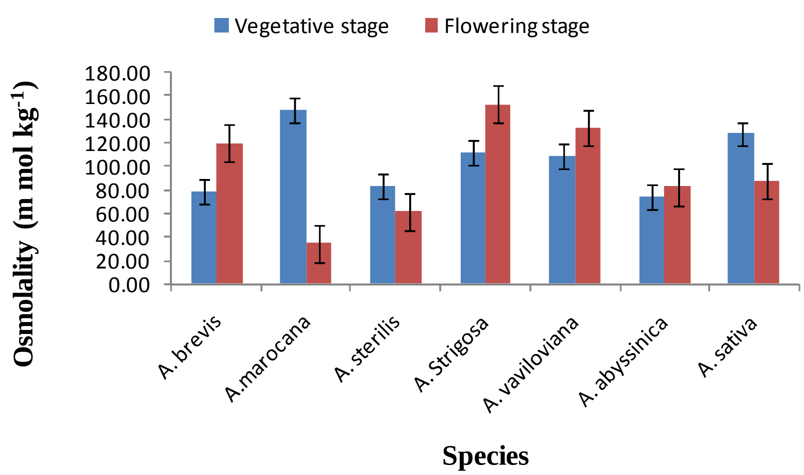


Fig. 4. Comparison of percent increase in osmolality over control in different *Avena* species under moisture stress at vegetative and flowering stages.

Similarly, in *A. abyssinica* the RWC value was reduced from 85.32 to 42.41% at a water potential value of -2.47 MPa, osmotic potential of -2.31 MPa, osmolality of 934 mmoleskg^{-1}, proline of 818 µmole g^{-1} fw at vegetative stage (Table 1) and at flowering stage the value was found to be 47.76%, -6.267 MPa, -2.470 MPa, 997 mmoleskg-1, 425.5 µmole g^{-1} fw for the same parameters (Table 2).

A. sativa showed maximum drought symptom on 9th day from the day of drought treatment. The RWC value decreased from 87.21% on the 1st day to 43.65% on the 9th day with a water potential value of -3.67 MPa, osmotic potential of -3.22 MPa, osmolality of 1298 mmoleskg^{-1}. The proline accumulation increased from an initial value of 12.40 µmole g^{-1} fw to 596 µmole g^{-1} fw (Table 1). At flowering stage, the maximum drought symptom occurred on the 5th day with the RWC value of 40.435% on the 5th day, water and osmotic potential value of -4.567 MPa and -2.932 MPa respectively. The proline accumulation was 31.2 µmole g^{-1} fw as compared to initial value 12.310 µmole g^{-1} fw (Table 2).

At vegetative stage, all the seven species of *Avena* grouped under three category according to the physiological responses produced due to moisture stress. In the group one, *A. sterilis* proved to be the highest tolerant species among the three, in terms of highest percentage of proline accumulation with a moderate level of osmolality concentration and RWC percentage. In the second group *A. strigosa* showed a maximum tolerance on up to 12th day with the highest magnitude of drought with minimum decrease in water and osmotic potential, higher accumulation of osmolality, less decrease in RWC and moderate accumulation of proline. *Avena sativa* is the most abundant cultivated species of *Avena* showed the extent of tolerance up to the 9th day only. Hence, *A. sterilis* can be considered as the highest tolerant species under drought situation followed by *A. strigosa* and *A. sativa* when there is water scarcity at vegetative stage. At flowering stage, drought condition is only extend up to 5th day in remaining six species of *Avena* (*A. strigosa, A. brevis, A. vaviloviana, A. abyssinica, A. maroccana* and *A. sativa*). Only one species, *A. sterilis* experienced drought till 4th day. The extent of drought period is less as compared to vegetative stage may be due to the increase in air temperature and higher biomass which is ultimately required more amount of water for its metabolic activity. Among this six species, *A. moroccana* showed better moisture retention capacity in terms of a lesser amount of decrease in RWC % with a reasonable increase in osmolality, proline, a lesser amount of decrease in water potential, osmotic potential followed by *A. abyssinica*, so that these species are considered to be in flowering stage for drought tolerance. In the present investigation, proline content was found to be increased due to corresponding to decrease in RWC, water potential, osmotic potential, increase in osmolality among these species as proline is a well known osmoregulator that accumulates under stress conditions particularly drought, salinity and osmotic stress. However, the extent of increase in proline varied among the species. It is concluded that higher accumulation of proline with a low RWC, water potential, osmotic potential and higher osmolality may have better capability to sustain water stress and may be considered as the tolerant species. This was with the agreement of the data reported for other species (Peuke *et al.*, 2002). In the *Avena* species, the link between leaf water potential and osmotic potential revealed active osmotic correction. This matches the findings of Steinberg *et al*., (1989), as seen in

Fig. 1. According to Yang *et al.,* 2011, osmotic adjustment (OA) is a fundamental feature of plant osmo-tolerance, defined as the decrease of osmotic potential in plant tissue owing to net accumulation of organic or mineral solutes. According to Bimpong *et al*., (2011), measuring relative water content (RWC) and water potential (WP) are markers of plant water status that may be used to track the progression of stress in plants developing under drought.

In general, when water stress increases, water potential decreases (more negative). The current study found that when the amount of the stress increased, the leaf water potential dropped. Moinuddin *et al.* (2004) observed similar results in chickpea. Morgan (1995) found comparable findings using wheat lines with varying osmoregulatory capacities. High proline concentration in cells has been linked to protein denaturation prevention (Kumar *et al.,*1994), enzyme structure and activity prevention (Samuel et al. 2000), and membrane protection from damage by reactive oxygen species (ROS) generated under drought and high light circumstances (Saradhi *et al*., 1995). Proline content in Avena species increased by several times as the amount of the stress increased. Different perspectives on the relevance of proline for evaluating stress reactions have been documented, although they are still conflicting (Hare and Cress 1997). Proline foliar spray improved drought stress resistance, which can be linked to considerable changes in physio-biochemical and anatomical aspects of oat plants caused by proline (Ghafoor *et al*., 2019). It has been demonstrated that drought tolerance is a quantitative feature in cereals, and RWC is an accurate technique for screening drought tolerance (Teulate *et al.,* 2003). Several studies have found that RWC decreases under severe water deficit stress circumstances (Yousfi *et al.,* 2010), and that osmotic adjustment increases drought tolerance in particular crop species (Turner 1986). Higher levels of antioxidant enzyme activity, reactive oxygen and major reductions in photosynthesis-related markers were suggested for oats at high altitudes (Jinqiu *et al*. 2021).

There are differences in osmotic adjustment abilities among species and plant parts (Wang *et al*., 1995). The findings of this study are consistent with those of Sayer *et al.* (2008), who found a link between retaining higher levels of leaf water potential and drought tolerance in durum wheat. According to Slama *et al*., (2011) and Bibi *et al*., (2010), osmotic potential might be employed as a drought tolerance selection criterion because it contributed the most to water stress of all drought characteristics. Proline content in durum plants achieved a high value under water deficiency stress, according to Vendruscolo *et al*., 2007. This finding supports recent research on durum wheat and bread wheat (Mekliche *et al*., 1992), which demonstrated the impact of water stress on RWC in several wheat cultivars. Wu *et al*., (2017) and Anwar *et al*., (2018) reported that Oats can make morphological and physiological changes to adapt to environments under abiotic stress.

CONCLUSION

Climate change is a current problem, and researchers are working to understand its influence on crop development and production, as well as to discover appropriate management solutions to maintain crop productivity in the face of climate change scenarios. Water stress is damaging to crop growth, and losses are variable during different phenophases, according to our research (vegetative and flowering stage). *Avena maroccana* had the best moisture retention ability among the seven *Avena* species, with a lower RWC percent, a modest rise in osmolality, proline, smaller drop in water potential, and osmotic potential, followed by *Avena abyssinica*. As a result, a comprehensive programme to measure the impacts of water stress on crop yield in various agro-ecologies and agri-production situations is becoming more necessary.

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