International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 2165-2167

Received: 04-07-2018
Accepted: 08-08-2018

Estimation of proline content in advance mutant lines of oat (*Avena sativa* L.) under water stress condition

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Abstract
In this study, we evaluated proline accumulation profiles in leaves of mutant oat (*Avena sativa* L.) plantlets under water stress. Oat plantlets were observed to accumulate proline in leaves, stems and leaves during water stress. The study is conducted in 30 mutant lines of oat along with three checks JO-1, JO 03-91 and Kent under water stress condition. Results indicated that mutant line has proline content of 36.71 (micro gram per gram) of fresh leaf weight, which is higher than check JO-1 that has proline content of 35.10 (micro gram per gram) of fresh weight leaf, so that it is advantageous to select the mutant line MJ9315-17 for water stress or drought condition.

Keywords: stress, drought, proline, mutant

Introduction
Proline has been recognized as a multi-functional molecule, accumulating in high concentrations in response to a variety of biotic and abiotic stresses. It is able to protect cells from damage by acting as both an osmotic agent and a radical scavenger. In plants, Proline accumulation has been reported to occur after salt, drought, high temperature, low temperature, heavy metal, pathogen infection, anaerobiosis, nutrient deficiency, atmospheric pollution and UV irradiation. The large accumulation of proline that occurs during drought may be explained in part by its basic chemical properties: proline is the most water soluble of the amino acids and exists much of the time in a zwitter ionic state having both weak negative and positive charges at the carboxylic acid and nitrogen groups, respectively. Proline shares this property with other compounds collectively referred to as "compatible solutes" that are accumulated by a wide range of organisms to adjust cellular osmolarity (Yancey, 2005) [8]. Pro accumulation is believed to play adaptive roles in plant stress tolerance. Pro has been proposed to act as a compatible osmolyte and to be a way to store carbon and nitrogen (Hare and Cress 1997) [2]. Salinity and drought are known to induce oxidative stress. Early *in vitro* studies showed that Proline can be a ROS scavenger. Many of these roles of proline have been discussed in recent reviews (Verbruggen and Hermans, 2008, Lehmann *et al*. 2010 and Szabados and Savoure 2010) [7, 4, 6].

Experimental Site
The experiment was carried out under All India Coordinated Research Project on Forage Crops, Department of Genetics & Plant Breeding at Seed Breeding Farm, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P).

Experimental Material
The experimental material comprises of 30 mutant lines of oat along with three checks JO-1, JO 03-91 and Kent.

Estimation of Proline
Proline accumulation under abiotic stress (moisture salinity) in various crop plants was reported and considered as a defense on mechanism. It is suggested to act as an osmolyte-Protectant as well as a source of nitrogen during recovery from stress.

Reagents

Sulphosalycylic acid (3%): Three gram of sulphosalycylic acid was dissolved in 100 ml of distil water.

Orthophosphoric acid (6N): Required volume of orthophosphoric acid (3.81 ml) was taken and volume was made to 100 ml, using distilled water to get 6 N orthophosphoric acid.

Acid Ninhydrin: Ninhydrin (1.25) was dissolved via blend of 30 ml of glacial acetic acid and 20 ml of 6 N Orthophosphoric acid.

Procedure

Leaf sample (0.5g) is homogenized in 5 ml of sulphosalycylic acid 3% using mortar pestle. The homogenate is filtered through what man No.1 filter paper and filtrate is collected, which is used for the estimation of proline content two ml of extract is taken in test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent was added the reaction mixture is heated in boiling water bath at 100 ^{0}C for 30 minutes. Brick red colour develops. After toluene added and then transferred in to a separating funnel. After through mixing, the chromophore containing toluene is separated and its absorbance read at 520 nm in spectrophotometer against toluene blank concentration of proline is estimated by referring to a standard curve made from known concentration of proline.

Calculation

Express the proline content on fresh-weight-basis as follows:

$$\text{m moles per g tissue} = \frac{\text{mg proline/mL x mL toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

Where, 115.5 is the molecular weight of proline.

Results and Discussion

Proline is a dominant organic molecule, acts as mediator osmotic adjustment or osmoregulation, a stabilizer of sub-cellular structure, and a sink for energy and even as a stress related signal molecule. The involvement of proline as a compatible solute in osmotic adjustment under water stress is well been documented.

MJ9315-17 mutant line accumulated the greatest mean quantity of (36.71µg g-1 FW leaf) which is greater than the check JO-1 (35.10 µg g-1 FW), followed by MJ9315-32 (31.61 µg g-1 FW leaf), MJ9315-46 (22.77 µg g-1 FW leaf), MJO15-11 (21.78 µg g-1 FW leaf), MK15-33 (20.50 µg g-1 FW leaf) and the lowest value was found in MJ9315-41 (0.54 µg g-1 FW leaf). Selection of the lines that has higher proline content is advantageous under drought conditions.

Higher proline accumulation in plants during stress is credited to maintenance of high turgor and continuous growth even under stress condition. Increased trend of proline content with moisture stress suggest its protective and stabilized role. These results were in conformity with the earlier findings of Mafakheri *et al*. 2010 [5], Caballero *et al*. 2005 [1] and Hu *et al.* 2004 [3].

Table 1: Proline content (µg proline g-1 FW leaf) of selected oat lines

S. No	Lines	Proline (micro gram per gram) of fresh leaf weight
1	JO-1 CHECK	35.10
2	MJO15-5	9.53
3	MO15-6	18.90
4	MJO15-7	1.86
5	MJO15-11	21.78
6	MJO15-14	11.00
7	MJO15-17	15.44
8	MJO15-21	1.80
9	MJO15-22	1.38
10	MJO15-25	1.22
11	MJO15-28	1.62
12	KENT CHECK	1.22
13	MK15-3	5.20
14	MK15-8	1.28
15	MK15-10	1.61
16	MK15-15	4.88
17	MK15-16	4.68
18	MK15-17	4.73
19	MK15-20	12.00
20	MK15-32	4.82
21	MK15-33	20.50
22	JO 03-91 CHECK	2.46
23	MJ9315-4	11.78
24	MJ9315-12	2.45
25	MJ9315-13	9.80
26	MJ9315-17	36.71
27	MJ9315-21	5.07
28	MJ9315-24	5.21
29	MJ9315-31	1.96
30	MJ9315-32	31.61
31	MJ9315-33	7.21
32	MJ9315-41	0.54
33	MJ9315-46	22.77

References

1. Caballero JT, Verdugeo CV, Galan J, Jimeneg ESD. Proline accumulation as a symptom of drought stress in maize. A tissue differentiation, requirement. J of Experimental Botany. 2005; 39(7):889-897.
2. Hare PD, Cress WA. Metabolic implications of stress induced proline accumulation in plants. Plant Growth Regulation. 1997; 21:79-102
3. Hu J, Jiang D, Cao W, Luo W. Effect of short-term drought on leaf water potential, photosynthesis and dry matter partitioning in paddy rice. Chin. J App. Ecol. 2004; 15:63-67.
4. Lehmann S, Funck D, Szabados L, Rentsch D. Proline metabolism and transport in plant development. Amino Acids. 2010; 39:949-962.
5. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi E. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Austrial Journal of Crop Science AJCS. 2010; 4(8):580-585.
6. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends in Plant Science. 2010; 15:89-97.
7. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids. 2008; 35:753-759.
8. Yancey PH. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology. 2005; 208:2819-2830.