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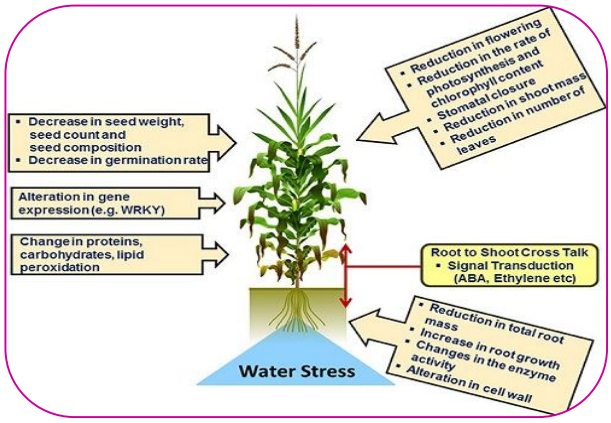
INFLUENCE OF WATER STRESS ON PROTEIN PROFILE ON PROMISING *SORGHUM* CULTIVARS



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ABSTRACT

The present investigation was aimed to study the influence of water stress on protein profile on promising *Sorghum* (*Sorghum bicolor*) cultivars e.g. RSV-669, RSV-626, RSV-623 and RSV-629 along with check RSLG-262, under different levels FC % (Field Capacity) at seedling as well as anthesis stage.

The drought tolerant promising cultivar RSV-669 has synthesized a new protein of 35.5 KD at 40% FC. The same band was also noted in another probable drought tolerant cultivar RSV-629 and RSV-626, but the intensity and width of band was less. The cultivar RSV-623 was also having this band but its intensity was very low, indicating its susceptible nature. The drought tolerant nature of promising cultivar RSV-669 is strengthened, because exactly similar type of band appeared in RSLG-262 (released drought tolerant) cultivar of sorghum. At 20% FC almost all the bands became very faint and invisible, indicating very high degradation of proteins at the extreme water stress level in all the cultivars. However the cultivars RSV-623 and RSV-669 both had shown new bands of very high intensity 17.5 KD, which indicate that these varieties should give comparatively better performance even under extreme drought condition.

KEYWORDS: *Sorghum bicolor, Water stress, Field Capacity, Protein Profile, SDS-PAGE.*

INTRODUCTION:

The most popular and economically important rainfed cereal crop *Sorghum bicolor* (L.) Moench, popularly called as "camel crop" because of its drought resistance, ranks 1st in the state of Maharashtra, 3rd in India and 5th in the world among the major food crops (Deshmukh Ravindra, 2017)

This crop is widely grown in the states like Maharashtra, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Rajasthan, Karnataka, Gujarat and Tamilnadu in both the seasons, i.e. *kharif* and *rabi*. In India, sorghum occupied an area of 6180000 hectares, which is highest in the world, with the production of 5280000 metric tons of grains and an average productivity of 854 kg ha^{-1}. In the state of Maharashtra, sorghum is the major cereal crop occupying an area of about 1935.3 thousand hectares with the annual production of 3162 thousand tons and an average productivity of 612 kg ha^{-1} (Deshmukh Ravindra, 2017).

Rabi sorghum predominately grown in the states of Maharashtra usually on the residual soil moisture and the yield is a function of *kharif* rains. Thus, *rabi* sorghum generally suffers from severe moisture stress. This situation totally disturbs the *rabi* production levels especially on light and medium soils, where grain and fodder yields are drastically reduced (Bapat and Gujar, 1990).

MATERIALS AND METHODS:

The present investigation planed for to study the impact of water stress induced different levels of field capacity(Deshmukh Ravindra, 2017) was studied in various promising cultivars of sorghum. The influence of water stress on protein profile was investigated. The pot culture experiments were conducted at the Department of Botany, Savitribai Phule Pune University. The authentic seeds of promising and released cultivars of sorghum were procured from the Senior Sorghum breeder, Sorghum Improvement Project, Mahatma Phule Agricultural University, Rahuri, Dist. Ahmednagar, (MS) for this investigation. The four promising cultivars i. e. RSV-669, RSV-626, RSV-623 and RSV-629 and check cultivar RSLG-262 were selected for this investigation.

SDS-PAGE ELECTROPHORESIS:-

Proteins of control and stressed plants were separated on 10% SDS-PAGE as per method described by Laemmli (1970). The leaves (0.2gm) were crushed in 2 ml of ice cold phosphate buffer (pH 7.0). Samples were spun at 15000 rpm at 4^0C for 15 min. Supernatants were taken in other tube and stored at -20^0C. These supernatants were used as protein samples for gels. The protein content of samples were quantified by method described by Lowery *et al.* (1951). Equal amount of proteins (30µg) were denatured in sample buffer at 100^0C in water bath and loaded in wells. Gels were run at 30mA and at constant voltage.

SILVER STAINING PROCEDURE:

Silver staining relies on differential reduction of silver ions that are bound to side chains of amino acids. Thus, free silver ions (Ag^{++}) are reduced to metallic silver on the surface of protein molecules. The metallic silver is deposited to give blackish brown bands. Silver staining is 100 times more sensitive than CBB staining, i.e. it can detect as little as 0.1 to 1.0 ng of polypeptide in a single band. The procedure of the same is summarized below.

1. After the completion of electrophoresis, protein gels were fixed by incubating the gel for 4-12 hours at room temperature with gentle shaking in a solution of ethanol : glacial acetic acid : water in ratio.
2. Fixing solution was discarded and the gels were transferred in 30% ethanol, shake gently for 30 min at room temperature.
3. Repeat step 2.
4. Ethanol was discarded and the gels were rinsed thoroughly with double distilled deionized water. The gels were incubated for 10 min at room temperature with gentle shaking.
5. Repeat step 4.
6. The gels soaked in a freshly prepared 0.02% sodium thiosulphate solution for 1 min.
7. The gels washed with double distilled deionized water for 1 min.
8. Water was discarded and, gels were transfer into 0.2% solution of silver nitrate for 30 min at room temperature with gentle shaking.
9. Silver nitrate solution was discarded and both sides of the gels were washed with deionized water.
10. The gels were transferred and developed in (2.5% sodium carbonate containing 0.02% formaldehyde). The gels were incubated at room temperature with gentle agitation. The gels were watched carefully. Stained bands of proteins should appear within a few minutes. Incubation was continued until the desired contrast is obtained.
11. The reaction was stopped by washing the gel in 1% acetic acid for few minutes. Then, the gels were washed several times with deionized water.
12. The gels were stored in 50% methanol.

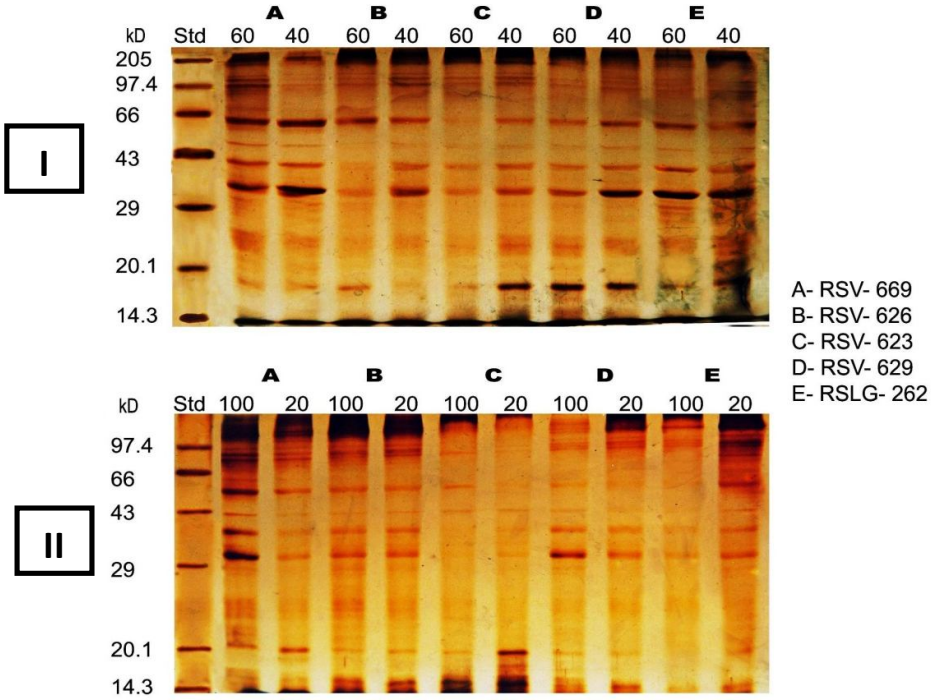
RESULTS AND DISCUSSION:

To confirm the drought tolerant or susceptible nature of the cultivars under investigation, their protein profile was studied with the aid of SDS-PAGE by using flag leaf at anthesis stage. The results shown in Photograph I and II indicated considerable differences in the protein profile pattern of the probable drought tolerant and susceptible cultivars at different levels of water stress, compared at 60 and 40% FC (Photograph I) as well as 100 and 20% FC (Photograph II) in all the cultivars like RSV-669, RSV-626, RSV-623, RSV-

629 and the check cultivar RSLG-262. Water stress causes both reductions in rate of protein synthesis as well as changes the type of proteins produced. These stress induced proteins allow plants to make biochemical and structural adjustments that enable the plants to cope up with the stress. Thus understanding the molecular as well as biochemical basis of drought tolerance will help in developing strategies for improving drought tolerance. Identification of molecular markers link to drought tolerance traits may provide plant breeders with a new tool for selecting cultivars with improved drought tolerance.

The present analysis will help to screen the drought tolerant or susceptible nature of the sorghum cultivars on the basis of molecular parameters. The protein profile shows some qualitative and quantitative differences.

Photograph I &II: Protein Profile of Sorghum cultivars



I & II: Protein profile of five selected cultivars of sorghum under different levels of FC % (20,40,60& 100) at anthesis stage.

From the Photograph I it is clearly observed that a very intense band is marked in probable drought tolerant promising cultivars RSV-669 having □35.5 KD at 40% FC. This band was showing maximum intensity in this cultivar supporting its drought tolerant nature, predicted on the basis of physiological, biochemical, enzymological, morphological and yield parameters. The same KD band is noticed in another probable drought tolerant cultivar RSV-629, but the intensity and width of band was slightly less than RSV-669. Compared to these two cultivars, lowest intensity and width of same band was noted in RSV-626, which was also considered as moderate drought tolerant cultivar on the basis of above mentioned parameters.

The cultivar RSV-623 showed very faint nature of the same band of the same KD, indicating its susceptible nature. The drought tolerant nature of RSV-669 on this band is strengthened, because of exactly similar type of band appeared in RSLG-262 (released drought tolerant) cultivar of sorghum.

The cultivars RSV-623, RSV-629 and even the check cultivar RSLG-262 showed all together different and a new band of □17.5 KD. However in RSV-669 and RSV-626 its intensity and width was comparatively less. The other bands showed the difference only in the intensity during the stress levels.

Jadhav *et al.* (2001) reported that the effect of seed treatment with abscisic acid and putrescine on drought tolerance of *rabi* sorghum cultivars and monitor the differences in SDS-PAGE electrophoretic pattern of soluble proteins. The soluble protein showed 10 to 11 bands of different molecular weights between 6 to 68 KD. They further noted that extra band of 12 protein subunits (33 KD) increased due to drought stress. In the present investigation also □36.5 KD band was induced in RSV-669, RSV-629 and RSV-626 with different intensity. The stress responsive proteins resolved from SDS-PAGE indicate that these newly synthesized protein under water stress might be providing drought tolerance to these cultivars, which may be called as dehydrin proteins as mentioned by Singh *et al.* (1987), Close *et al.* (1993). Ahire *et al.* (2005) noted a newly synthesized protein band of □17.78 KD size in drought tolerant cultivar of chick pea. Tyagi *et al.* (1995) also reported a polypeptide band □22 KD in *Lathyrus sativus*. Similarly Close *et al.* (1993) identified a dehydrin protein of □17 KD size in drought tolerant maize. In the present investigation also the sorghum cultivars RSV-629 and RSV-623 had shown same size of band (□17.78 KD). Similarly Lee *et al.* (2002) also reported a 18 KD band as a major protein band induced by drought stress in white clover. Thus the results obtained in present investigation are in agreement with a result reported by earlier researchers.

According Shinozaki and Yamaguchi- shinozaki (1997) genes induced during drought stress thought to function not only in protecting the cell from water deficit by the production of important metabolites but also in the regulation of genes for signal transduction in drought stress response.

The Photograph I clearly indicate that another new protein band □35.5 KD is synthesized under water stress condition (60 and 40% FC) in the cultivars RSV-669, RSV-629, RSV-626 and in check cultivar RSLG-262. It appeared even in RSV-623 but its intensity was very less.

The highly intense nature of this band in RSV-669 clearly indicates its drought tolerant nature because the intensity of band in this cultivar was exactly similar to the band noted in drought tolerant check cultivar RSLG-262. The intensity of band in RSV-669 was followed by RSV-629 which we have categorized as moderate drought tolerant cultivar of sorghum. The results of biochemical, physiological and morphological as well as yield parameters are highly supportive to the conclusion drawn on the basis of molecular studies and *vice versa.* The moderately drought tolerant cultivar RSV-626 follows the cultivar RSV-629 while the probable susceptible cultivar RSV-623 had very less band intensity of this type of protein.

Ahire *et al.* (2005) recorded a newly synthesized protein band of □36.3 KD in drought tolerant chick pea cultivar. The results of the present investigation are in agreement with the above finding.

Dure *et al.* (1989) postulated that such type of newly synthesized protein may be belonging to a family of hydrophilic proteins, which play a role in the acquisition of desiccation tolerance or water stress tolerance. These stress induced proteins are confirming the probable drought tolerant, moderately drought tolerant and susceptible nature of the promising cultivars of sorghum investigated in the present study.

The occasional better performance in response to water stress shown by RSV-623 may be assigned to the additional protein band of 17.5 KD recorded in it, but it requires further confirmation.

The Photograph II shows the comparison of different protein bands at 100% FC and 20 %FC, from the same photograph it was noted that at 20% FC almost all the bands become very faint and as good as invisible. Indicating very high degradation of proteins at the extreme water stress level. However the cultivars RSV-623 and RSV-669 had shown few bands of very high intensity □17.5 KD which indicate that these varieties should give comparatively better performance even under extreme drought conditions, if supported by physiological, biochemical, enzymological, morphological and yield parameters.

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