Chapter-I
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1.1 Scenario of drought at global and Indian level:-

Throughout the world the arid and semiarid regions are drastically influenced by the soil moisture deficit situations year after year. Out of the total 1474 m ha arable lands world over, the unirrigated rainfed area is about 84% (1247 m ha). Hence major attention should be given to the crops grown in this area, because environmental stresses including water stress reduce crop yields in this region from 51 – 82% (Bray et al., 2000). Same is the situation in India, in which the total arable land is only 143.8 m ha, of which, the area under rainfed takes the lions share (65.5 m ha), followed by dry land (34.5 m ha). The area under arid and semi arid zones constitute 1.37 m Km$^2$, of which, the area under arid zone is 29.2 percent (0.40 m Km$^2$) and semi arid zones is 70.8 per cent (0.97 m Km$^2$). Of all the states, the area under arid zone is maximum in Rajasthan (61%) followed by Gujrat (19.5%). Whereas, the area under semi arid zone is maximum in Maharashtra (19%) followed by Karnataka and Andhra Pradesh (15%). Like many other states the agriculture in Maharashtra, the third largest state in India in terms of area and production, is rainfed in nature. Only 15% of its cultivated area is under irrigation.

The response of plants to the abiotic stresses is highly variable due to its compound nature. The cell, tissue and plant responses to abiotic stress factors involve several mechanisms simultaneously. It is essentially a multigenic response, involving several biochemical and physiological attributes. But still today the knowledge of biochemistry and physiology of plant abiotic stresses is not completely understood (Grover et al., 2004). This comes in the way of using targeted approach in breeding for abiotic stress tolerance. The intensity of abiotic stresses is by and large on the rise due to intensive farming being practiced world over, or due to other man made reasons. Hence this major aspect of screening for drought tolerance on the basis of physiological and biochemical approaches in one of the major rainfed crop like sorghum was under taken in the present study.

The resistance or tolerance to drought stress in sorghum can be manifested, identified, measured and studied at cellular, tissue, organ, whole plant or at the level of the crop community. Any response that constitutes resistance to be practically usable in breeding needs to be manifested as an advantage in survival during stress, or recovery after alleviation of stress thus resulting in better crop stand in the field.
1.2 Impact of water stress on crop physiology and yield:

Indian agriculture even today is a gamble in the monsoon (Swaminathan, 2004), which is closely linked to the annual precipitation pattern. About two third of the cultivable area is rainfed (Venkateswarlu, 2004). The adequate production of staple grains in India and even in many other countries is mainly threatened by water stress, which reduces more than 50% of the grain yield in cereals. In agricultural country like India over one half of the cereal diet is derived from sorghum, per millet, wheat, rice, maize etc. Rice, sorghum and wheat are the main food, feed and forage crops of the world, hence their production should be increased by 50% to fulfill the demand of expected 8 billions world population by 2025. But it seems to be impossible because abiotic stress like drought takes a serious toll of the food grains; hence search for the water stress tolerant crop genotypes is an urgent need of the time. For this thorough understanding of various physiological, biochemical, enzymological and molecular processes under stress conditions will be a great boon in the endeavor to provide food security to all.

The information regarding survival and yield potential of various agricultural crops under stress conditions, their response to stress mechanisms adopted for stress tolerance, early vigor, morphology of roots, canopy structure and leaf architecture, osmoregulation, lipid peroxidation, membrane damages, antioxidant enzymes, RWC, carbon isotope discrimination, photosynthetic rate, mineral nutrition, partitioning of assimilates, delayed senescence, remobilization of reserves etc. will help the researchers to obtain the drought tolerant cultivars.

The effects of drought on crop plants are complex and variable and are greatly accentuated by a number of interacting factors. The onset of drought in general has been observed to reduce seed germination, emergence, hypocotyls length, water uptake and the mobilization of dry matter reserves even at the early growth stages. In addition to differential accumulation of carbohydrates, drought stress causes a marked reduction in leaf area. Soil moisture availability at the seed germination, anthesis and at post anthesis (grain filling and ear head formation) growth stages in various crops will determine to what extent the production potential of crops would be expected in drought prone situations.

The yield loses in field crops may be attributed to moisture stress at the critical sensitive growth stages (Boyer, 1996). Crops therefore differ in their susceptibility of growth stages to water stress. The stress exposed plants immediately lower down the RWC, leaf water potential, osmotic potential and turgor pressure. Hence to improve the grain production under water stress condition, the plants have to undergo various physiological and biochemical adaptations to maintain the turgor pressure.
1.3 Drought tolerance:

According to Grover et al. (2004) plant tolerance to soil moisture deficit is an important adaptive feature, which is achieved in some of the following ways.

i) Proportional and sequential growth retardation,

ii) Diversion of carbohydrates to required portions,

iii) Physiological potentiality, synthesis and accumulation of certain biochemical compounds,

iv) Synthesis and accumulation of specific proteins,

v) Accumulation of osmolytes and other complex processes,

vi) Morphological adaptation such as early flowering, decreased plant height, reduction in leaf area and dry weight and increased leaf conductance,

vii) Maintenance of turgor pressure,

viii) Osmotic adjustments through accumulation of polyols, amino acids (such as proline) and amino acid derivatives such as GBs to restore turgor pressure,

ix) Integrity and stabilization of cell membrane structure.

1.4 Selection indices for drought tolerance:

According to Grover et al. (2004) for the successful selection and evaluation of large number of germplasm lines and segregating materials or population, the consideration of phenological and morphological traits showing direct correlation with high drought tolerance concomitant with high productivity are desired. However, such parameters are few and not universal in application. But the most common and convenient ones include: FWt and DWt of whole plant, seed germination, days to flowering, leaf expansion, 1000 seed weight, seeds per pod, seed yield, chlorophyll stability index, stomatal conductance, osmoregulation, turgor pressure, leaf water potential, accumulation of solutes and specific proteins etc.

According to the current views drought tolerance includes three major events; such as osmotic adjustment, antioxidant capacity and desiccation tolerance (Klueva et al., 1996). Osmotic adjustment results from the accumulation of compatible solutes within cells, which lowers the osmotic potential and helps to maintain the cell turgor. It is associated with sustained plant production and yield under drought stress in several crops including sorghum (Ludlow, 1993). As suggested by Bohnert and Jensen, (1996) the accumulated osmotic solutes also protect the cell membrane integrity, prevents inactivation of enzymes, and alleviates protein denaturation and aggregation.

Antioxidant capacity is the ability of plants to detoxify reactive oxygen radicals (ROS) produced under stress, which provides structural tolerance under stress. While the
desiccation tolerance is the capacity of plants, to survive in low leaf water status through important mechanisms other than osmotic adjustments and antioxidant capacity, for example formation of drought induced proteins of LEA family. These proteins play a major role as desiccation protectant (Ingram and Bartels, 1996).

The understanding of physiological basis of stress resistance and an advent of molecular technologies allowed the plant breeders and biotechnologists to address this problem much more efficiently than in the past, as a result of this various abiotic stress tolerant traits were successfully introduced in to several crops either through hybridization or transgenic methods.

Crop production is hindered by various stresses encountered by plants in the field. Abiotic stress like drought disturbs numerous biochemical and physiological processes in plant cells (Grover et al., 2004), hence research work on these aspects is necessary to elucidate molecular mechanisms employed by plants to combat environmental stresses, to pinpoint important genes and to determine the bottle necks of plant resistance to drought.

The physiological and biochemical mechanisms of abiotic stress resistance in various plants have been explained by Levitt (1980), Ludlow and Muchow (1990), Ludlow (1993), Fukai and Cooper (1995), McKersie and Leshem (1994), Bohnert et al. (1995) and Boyer (1996). They have further investigated the drought tolerant potential of various plants, breeding for stress environments, physiological attributes associated with drought resistance, development of drought resistant cultivars using morpho-physiological traits, varietal response to environmental stress, critical evaluation of plants for improving crop yields in water limited environments, stress and stress cropping in different cultivated plants.

It is evident that there is no single character either morphological, physiological or biochemical which can lead to selection for drought resistance. However, one can concentrate on the characters which bear close relation with yield stability under changing stress environments, since yield formation is a consequence of several steps involving many phases of plant growth and development (Chetti, 2002).

1.5 About the crop sorghum and impact of water stress on it:-

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 Table 1.1c and 5th in the world among the major food crops (Verma and Anand Kumar, 2005)

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* (Fig. 1.1). In India, sorghum occupied an area of 9.90 million hectares, which is highest in the world, with the production of 8.0 million metric tons of grains and an average productivity of 774 kg ha\(^{-1}\) (Table 1.1a). In the state of Maharashtra, sorghum is the major cereal crop occupying an area of about 4,756.1 thousand hectares with the annual production of 3,623.3 thousand tons (Anonymous, 2005), out of which about 1,332.9 thousand hectares were sown in *kharif*, while 3,223.2 thousand hectares in *rabi* season of 2004-05 (Anonymous, 2005). In the state of Maharashtra during the *kharif* and *rabi* season of 2004-2005 (Table 1.1b). Pune division including districts like Pune, Satara, Sangli and Kolhapur occupied first rank for the area under cultivation of sorghum (1,673.7 thousand ha), which was followed by Latur and Aurangabad divisions. Regarding total grain production (Table 1.1b) Latur division stood first (809.2 thousand tones), which was followed by Pune (797.5 thousand tones) and Kolhapur division (561.9 thousand tones).

*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).

The various constraints identified in the production of *rabi* sorghum are abiotic factors *viz.*., drought, temperature, salt, heat, and cold stress (Chen and Murata, 2002). Among these, drought is one of the major abiotic stress limiting crop growth and production under rainfed farming. Therefore in rainfed agriculture, there seems to be limited options to increase the area under irrigation and the only option is to improve the yield under water deficit conditions. Although drought management has been an option to increase realizable yields, it is now increasingly clear that the genetic improvement of drought tolerance is a more rewarding option (Yadav *et al.*, 2003). The intensity of drought stress is very important in sorghum production in arid and semi-arid regions of the world. Most areas of sorghum grain production world wide are under dry land conditions. If the drought stress on the plant becomes more severe, the plant encounters stunted growth and development, adversely affecting the reproductivity and even die (Chen and Murata, 2002). Consequently, plants have evolved peculiar ways of reacting to such stresses that constitute general and certain specific responses. (Chetti and Hiremath, 2002). Although the nature of stress induced changes in a plant depends much on the extent and speed of imposition of stress, basically these changes must begin with stress perception and signaling, followed by a change either directly in the membrane component or brought about through mediation of genes (Beyel and Brüggemann, 2005).
Table 1.1a: Area, production and yield of sorghum from 2000-01 to 2003-04 in India*

<table>
<thead>
<tr>
<th>Year</th>
<th>Area</th>
<th>Production</th>
<th>Yield</th>
<th>% coverage under irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-01</td>
<td>9.86</td>
<td>7.53</td>
<td>764</td>
<td>7.9</td>
</tr>
<tr>
<td>2001-02</td>
<td>9.80</td>
<td>7.56</td>
<td>771</td>
<td>NA</td>
</tr>
<tr>
<td>2002-03</td>
<td>9.20</td>
<td>7.08</td>
<td>769</td>
<td>NA</td>
</tr>
<tr>
<td>2003-04</td>
<td>9.49</td>
<td>7.33</td>
<td>772</td>
<td>NA</td>
</tr>
<tr>
<td>2004-05</td>
<td>9.90</td>
<td>8.00</td>
<td>774</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Area- Million hectares, Production- Million tonnes, Yield- Kg ha<sup>1</sup>

Table 1.1b: Sorghum producing divisions in Maharashtra: Area, Production and Productivity *

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabi sorghum</td>
<td>Total sorghum (kharif &amp; Rabi)</td>
<td>Rabi sorghum</td>
</tr>
<tr>
<td></td>
<td>Area</td>
<td>Prodn</td>
<td>Prodty</td>
</tr>
<tr>
<td>1</td>
<td>Konkan Dn</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>2</td>
<td>Nasik Dn</td>
<td>759</td>
<td>869</td>
</tr>
<tr>
<td>3</td>
<td>Pune DN</td>
<td>13545</td>
<td>1475</td>
</tr>
<tr>
<td>4</td>
<td>Kolhapur Dn</td>
<td>2291</td>
<td>891</td>
</tr>
<tr>
<td>5</td>
<td>Aurangabad Dn</td>
<td>5776</td>
<td>2091</td>
</tr>
<tr>
<td>6</td>
<td>Latur Dn</td>
<td>5442</td>
<td>2079</td>
</tr>
<tr>
<td>7</td>
<td>Amravati Dn</td>
<td>226</td>
<td>169</td>
</tr>
<tr>
<td>8</td>
<td>Nagpur Dn</td>
<td>500</td>
<td>199</td>
</tr>
<tr>
<td>9</td>
<td>State Total</td>
<td>28539</td>
<td>7773</td>
</tr>
</tbody>
</table>

*Area in "00" ha, Production in "00" tonnes, Productivity in Kg ha<sup>1</sup>

Table 1.1c: Food grain production in India
(in million tonnes)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Years</th>
<th>2000-01</th>
<th>2001-02</th>
<th>2002-03</th>
<th>2003-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td></td>
<td>84.98</td>
<td>93.34</td>
<td>72.66</td>
<td>87.94</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>69.68</td>
<td>72.77</td>
<td>65.10</td>
<td>76.12</td>
</tr>
<tr>
<td>Jowar</td>
<td></td>
<td>07.53</td>
<td>07.56</td>
<td>07.08</td>
<td>07.36</td>
</tr>
<tr>
<td>Cereals</td>
<td></td>
<td>185.74</td>
<td>199.48</td>
<td>163.05</td>
<td>197.78</td>
</tr>
</tbody>
</table>
Fig. 1.1 Map showing area of cultivation of sorghum
Water stress increases the variable response observed in biochemical constituents and metabolism in case of mild, moderate and severe water stress. Thus, protein and chlorophyll biosynthesis are sensitive to rather mild stress, whereas under condition of moderate stress, nitrate reductase level, growth hormone metabolism and carbon dioxide assimilation begin to be affect. Moderate to severe stress is associated with serious disruption of cell metabolism as indicated by increase in respiration and the accumulation of proline and sugars (Matysik et al., 2002). Plants adapt to survive and grow in the presence of environmental stresses since growth and defense against environmental stress, rely on energy release from metabolic substrates (Smith et al., 2000).

Several drought tolerant varieties of sorghum have been identified on the basis of their ability to give good yields under drought conditions in the field. Since photosynthesis has long term consequences on crop yield, adaptation of the photosynthetic apparatus to withstand drought stress would constitute an important stress tolerance mechanism.

In the present investigation quantitative information on various biochemical, physiological, enzymological, morpho-physiological and molecular changes associated with moisture stress in four different promising genotypes of sorghum during the various growth stages have been carefully analyzed to explore their drought tolerant or susceptible nature.

To cope with high stress sorghum plants accumulate soluble sugars, proline, glycine betaine, and total and de-novo proteins. These plants also synthesize various antioxidant enzymes like peroxidase, superoxide dismutase, catalase etc. which enable them to adapt with the moisture stress. Investigations of these mechanisms will assist the breeders and biotechnologists to manipulate the techniques for genetic improvement in these crops to sustain drought (Maiti et al., 2000).

1.6 About the morphology, agronomy and cultural practices of sorghum:-

Morphology:-

It is an erect plant that grows to a height of 0.5m- 4.5m, depending on the type of cultivar. It belongs to the sub family of panicoideae and tribe andropogoneae. *Sorghum bicolor* represents all the cultivated forms, while wild sorghum, and grass sorghum are either perennial weeds or grown as forage.

Sorghum is also commonly referred as kafir corn, milo, sorgos, durra, guinea millet or jowar.

Origin and History

The origin of sorghum is generally believed to be around the present day Ethiopia. Many authors now regard Ethiopian highlands as a primary center of domestication. From Ethiopia sorghum was taken to West Africa across the Sudan from where it was first grown among the Mande people of the upper Niger. Also from Ethiopia, sorghum was taken to East
Africa from where it was distributed among the Nilotic and Bantu people. Sorghum was taken from East Africa to India during first millennium from where it was taken to China in the early Christian era. Sorghum races in India are closely related to those in northeast Africa. From West Africa, sorghum was distributed to USA and other parts of the world through slave trade around mid 19th century.

**Distribution:-**

Main center of distribution of cultivated sorghums is in Africa, having been cultivated in Ethiopia for more than 5,000 years; possibly cultivated sorghums were also developed independently in India and China. Now sorghums are widely distributed throughout tropics, subtropics and warm temperate areas of the world (James Duke, 1983). Eighty per cent of the area devoted to sorghum is located within Africa and Asia, with average yields of 810 and 1150 kg/H.

The major sorghum producing countries in the world are USA and India followed by other countries like Latin America, Africa, Asia and Australia. Sorghum production increased steadily during 1960’s and 1970’s but after that it decreased significantly.

In Maharashtra Jowar is grown in all the districts except the coastal districts of Thane, Raigad, Ratnagiri and Sindhudurg (Table 1.1b and Fig. 1.1). The districts like Nashik, Dhule, Nandurbar, Jalgaon, Ahmednagar, Pune, Solapur, Satara, Sangli, Kolhapur, Aurangabad, Jalna, Beed, Latur, Osmanabad, Nanded, Parbhani, Hingoli, Buldhana, Akola, Washim, Amarawati, Yavatmal, Wardha, Nagpur, Bhandara, Gondia, Chandrapur, and Gadchiroli have large area under Kharif and Rabi cultivation (Anonymous, 2005).

**Agronomic conditions:-**

Sorghum can grow in a wide range of agronomic conditions and can still yield well even under unfavorable conditions of drought stress and high temperatures. It is generally grown between 40° North and 40° South of the equator, in warm and hot countries, characteristics of the semi-arid environment. Sorghum is usually grown in areas that are too hot and dry for maize.

**Soil:-**

Sorghum can tolerate a wider range of soil conditions. It does well both in fertile valley bottom soils as well as in nutrient poor soils. However, under dry land conditions, dry soils retard uptake of nutrients. The water content, physical resistance and porosity of soil usually affect growth and distribution of sorghum roots. Sorghum is very moderate in concern of soil types.
Photoperiod:-

Sorghum is short day plant and different cultivars vary in their sensitivity to the photoperiod. Sensitivity to the photoperiod is a genetically controlled character, which can be breed or select for. In West Africa, informal selection by farmers for day length sensitivity in sorghum resulted in crops that mature as the available water is exhausted in the early part of the dry season. This ensures that the crop fully utilizes the growing seasons, while avoiding diseases associated with high humidity during grain maturation.

Fertilizer Requirement:-

Fertilizer requirement for Jowar cultivation differs from season to season. Jowar is heavy surface feeder and exhausts the fertility of the soil. For a rainfed crop 6-8 tons of FYM or compost hectare$^{-1}$ and for irrigated Jowar, 8-12 tons of FYM hectare$^{-1}$ should be added to the soil before sowing. For good crop of sorghum fertigation is essential in rainfed area. The first dose of N and P should be applied @ 37.5 Kg hectare$^{-1}$ along with 35 kg of K hectare$^{-1}$.

Irrigation:-

Jowar can not tolerate water lodging, though it can withstand drought conditions to some extent. It needs about 50 cm of well-distributed rainfall for good yield. Under the irrigated condition in medium deep and deep soils, three irrigations are necessary at seedling, anthesis and milky stage for desirable yields.

Economic importance:-

Cultivated sorghum is grown chiefly for its grain which forms an important staple food in many countries of Africa and Asia, including India. In USA and other advanced countries, sorghum grain is, however, used chiefly as a feed for poultry, cattle and other animals. In some countries it is also a source of alcoholic beverages. Sorghum plants are widely used as fodder, either green or as hay and silage. Some cultivars are rich in sugar (sweet sorghums), and their juice is used for making jaggary in India and syrup in America. Under certain conditions of cultivation and certain stages of growth, few cultivars of sorghum contain poisonous cyanogenic glycoside known as dhurrin, which release hydrocyanic acid on hydrolysis, leading to occasional poisoning of animals fed on them.

Plant bases are an important source of fuel for cooking and the stems of wild varieties are used to make baskets or fish traps. (Frederiksen, 2003).

Nutritional values of sorghum grain:-

The various constituents and nutrient values of sorghum grains are given below. Water: 9.20 g; Energy: 339.00 kcal; Protein: 11.30 g; Total lipid: 3.30 g; Carbohydrate: 74.60 g; Ash: 1.57 g; Calcium: 110.00 mg; Iron: 4.00 mg; Phosphorus: 287.00 mg; Potassium: 350 mg; Sodium: 4.00 mg; Thiamine: 1.00 mg; Riboflavin: 0.40 mg;
1.7 The main objectives of this study:-

The present investigation was aimed to study the various physiological, biochemical, enzymological, molecular, morpho-physiological, growth and yield parameters in four promising cultivars of sorghum e. g. RSV-669, RSV-626, RSV-623 and RSV-629 along with check cultivar RSLG-262, during different levels of PEG induced water stress and FC % at the seedling as well as anthesis stage. The main objectives of the present investigation are given below:

- To understand the process of seed germination, seedling growth and biochemical constituents during seed germination.
- To study the contents of proline, glycine betaine, soluble proteins, free amino acids, reducing sugars, starch, phenols and mineral continuants.
- To investigate the relative leaf water content, membrane stability index, lipid peroxidation, chlorophylls, and carotenoids.
- To examine genetic variations in carbon isotope discrimination ($\Delta^{13}$C) values to know the WUE.
- To determine the activities of PEP Case and nitrate reductase enzymes.
- To investigate the role of antioxidant enzymes like SOD, PER and PPO.
- To study the protein profile in germinating seeds and in leaf at anthesis stage.
- To analyse the morphological, growth and yield parameters.