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BIOCHEMICAL CHARACTERIZATION OF DIFFERENT SEED VARIETIES OF RICE, WHEAT AND MUSTARD UNDER DIFFERENT LEVELS OF SALINITY STRESS

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Abstract: Salinity is one of the most important abiotic stresses limiting crop production in arid and semiarid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching. Salinity affects many morphological, physiological and biochemical processes, including seed germination, plant growth, and water and nutrient uptake. Two varieties of each Rice (CSR-10, VSR-156), Wheat (HD-2009 Kharchia-65) and Mustard (Kranti, CS-54) were selected and the experiment was conducted in Petri dishes of size 9cm diameter. All the genotypes were biochemically characterized for their relative salt tolerance under salinity stress at germination and seedling emergence in solution culture at Control 8, 12dSm^{-1} salinity levels by estimation of total soluble sugar, free amino acid and starch. Among these genotypes CSR-10, Kharchia-65, CS-54 has performed better under salinity levels at 8 and 12dSm^{-1}.

Keywords: Salinity, Abiotic, Rice, Wheat, Mustard, biochemical.

Introduction

Crops are often exposed to salinity immediately after planting in saline soil or in areas inundated by sea water or irrigated with brackish water. Nutritional imbalance caused by such ions leads to reduction in photosynthetic efficiency and other physiological disorders [1, 2]. Abiotic stresses including drought and salinity are currently the major factors which reduce crop productivity world-wide. Excessive amounts of salts in soil severely reduced the seed germination and further seedling growth and this has been well documented [3-6]. This has been ascribed due to salt-induced osmotic stress or due to its toxic effects or combination of both of these [3, 5, 6]. Salinization caused by intensive agriculture [7] have detrimental effects on germination of seeds [8, 9] and plant growth [10]. Salinity is one of the major limiting factors to the agricultural productivity; it constrains productivity nearly 20% of the cultivated area and half of the irrigated area worldwide. It also restricts agricultural expansion into areas that presently are not cultivated. The soils of arid regions of the world are frequently associated with high levels of ionic salts such as NaCl, Na_2SO_4, $CaCl_2$.These salts are normally contained in soils, but in regions of moderate to high rainfall, they are leached

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from the soil into the ground water. A complication in arid region is that of poor quality underground water for irrigation, which gradually adds even more salts to the soil. The soil salinity may cause several deleterious effects on growth and development of plants at physiological and biochemical level. Seed germination, seedling emergence and early survivals are particularly sensitive to substrate salinity as compared to the later development stages. The present study aims to exploit salt tolerance potential and genotype identification of Rice, Wheat and Mustard germplasm at initial growth stages, which may solve the problem of poor plant stand resulting from lower seedling emergence under salt affected areas.

Material and Methods

Two high yielding varieties of each Rice (CSR-10, VSR -156), Wheat (Kharchia- 65, HD-2009), Mustard (CS-54, Kranti), were selected and the experiment was conducted in Petri dishes of size 9cm diameter. All the genotypes were characterized for their relative salt tolerances under salinity stress at germination and seedling emergence stages in solution culture at Control, 8, 12dSm^{-1} salinity levels. Seeds were surface sterilized with 0.1% mercuric chloride solution for one minute, washed with distilled water and soaked in filter paper Whatman no.1 to remove excess water. Salt solution was changed every day to avoid increase in concentration due to evaporation.

The following observations were recorded:

1. Total soluble sugar
2. Free amino acid
3. Starch

Total soluble sugar

The total soluble sugar was determined by the method of Yemn and Willis (1954) [11].100 mg of oven dried material was taken and homogenized in 4ml of 80% ethanol and shifted to test tubes for heating in boiling water bath for 15 min. The material was centrifuged at 3000 rpm for 15 min. After discarding the supernatant, the residue was washed twice with 2 ml of 80 % ethanol each time and recentrifuged. The supernatant was pooled and final volume was made to 10 ml.0.2 ml of aliquot was evaporated to dryness in test tubes and after cooling, the residue was dissolved in 1ml distilled water. After adding 4ml of anthrone reagent (0.2g anthrone in 100 ml conc. H_2SO_4) the solution was heated in boiling water bath for 10 minutes. After cooling, optical density was noticed determined at 620 nm against blank (80% ethanol).

Free amino acid

Free amino acid was estimated according to Lee (1966) [12].

Reagents

Solution A: 1% ninhydrin in 0.1m citrate buffer (pH5.5)

Solution B: pure glycerol

Solution C: 0.1M citrate buffer (pH5.5)

Solution A, B and C were mixed in the ratio 5:12:2 (ninhydrin reagent)

Procedure

5ml of Ninhydrin reagent were added to 0.2ml of the above extract. The contents were shaken vigorously and pH was adjusted to 6.0 with sodium citrate. The mixture was taken heated in boiling water bath for 12 minutes. The tubes were cooled under running water to room temperature and optical density was recorded at 570nm. The blank was prepared by adding 5ml Ninhydrin reagent to 0.2ml of 80% ethanol.

Starch

Starch content was determined according to method of Hassid and Neufeid (1964) [13].

Extraction

6.5 ml chilled 56% perchloric acid was added to pellets which were left after extracting soluble sugar and the mixture was kept overnight under low temperature conditions, then centrifuged at 5000 rpm for 5 min. The supernatant was used for starch estimation.

Reagents

Anthrone reagent: As given in the estimation of total soluble sugars.

Procedure

0.2 ml of supernatant was taken and dissolved in 1ml distilled water per test tube. 4 ml of anthrone reagent was added and mixture was heated in boiling water bath for 10 min. After cooling at room temperature optical density was taken at 620 nm against blank.

Results and Discussion

Salt tolerance potential of Rice, Wheat and Mustard germplasms were evaluated at varying salinity stress (Control, 8, 12dSm^{-1}) and total soluble sugar, free amino acid and starch were recorded as shown in table 1.

Table 1. Biochemical parameters of rice, wheat and mustard under different levels of salinity stress

Variety	Treatment EC (dsm^{-1})	Total soluble sugar (mg/gdw)	Free amino acid (mg/gdw)	Starch (mg/gdw)
Rice:CSR-10	Control	25.6	25.6	273.3
	8	24.3	29.6	205.0
	12	23.5	38.5	107.5
Rice:VSR-156	Control	23.0	23.5	167.5
	8	21.0	25.5	160.5
	12	20.0	33.0	140.0
Wheat:HD-2009	Control	33.1	57.3	315.0
	8	32.0	80.6	225.0
	12	30.6	86.3	163.3
Wheat:Kharchia-65	Control	30.3	72.0	276.6
	8	29.1	84.0	181.6
	12	28.3	102.6	178.3
Mustard: Kranti	Control	7.6	61.5	350.0
	8	6.0	82.5	165.0
	12	5.0	93.5	162.5
Mustard: CS-54	Control	6.8	68.0	255.0
	8	5.5	109.5	233.3
	12	4.3	110.0	112.5

Genotypic varieties of Rice(CSR-10,VSR-156), Wheat (HD-2009, Kharchia-65) and Mustard (Kranti, CS-54) were biochemically characterized for their relative salt tolerance under salinity stress at germination and seedling emergence in solution culture at Control, 8, 12dSm^{-1}salinity levels and following observations were recorded total soluble sugar, free amino acid and starch. The CSR-10 variety under (Control, 8, 12dSm^{-1}) treatment show Total soluble sugar(mg/gdw)-25.6,24.3,23.5 followed by Free amino acid (mg/gdw) -25.6, 29.6, 38.5 and Starch (mg/gdw)-273.3, 205.0, 107.5.The VSR-156 variety under (Control,8,12dSm^{-1}) treatment show Total soluble sugar(mg/gdw)-23.0,21.0,20.0 followed by Free amino acid (mg/gdw) -23.5,25.5, 33.0 and Starch (mg/gdw)-167.5, 160.5, 140.0. The HD-2009 variety under (Control, 8, 12dSm^{-1}) treatment show Total soluble sugar (mg/gdw)-33.1,32.0,30.6 followed by Free amino acid (mg/gdw) -57.3,80.6,86.3 and Starch (mg/gdw)-315.0,225.0, 163.3.The Kharchia-65 variety under (Control, 8, 12dSm^{-1}) treatment show Total soluble sugar(mg/gdw)- 30.3,29.1,28.3 followed by Free amino acid (mg/gdw) – 72.0,84.0,102.6 and Starch (mg/gdw)- 276.6, 181.6, 178.3. The Kranti variety under (Control, 8, 12dSm^{-1}) treatment show Total soluble sugar (mg/gdw)-7.6, 6.0, 5.0 followed by Free amino acid (mg/gdw) – 61.5,82.5,93.5 and Starch (mg/gdw)- 350.0, 165.0, 162.5.The CS-54 variety

under (Control, 8, 12dSm^{-1}) treatment show Total soluble sugar (mg/gdw)-6.8, 5.5, 4.3 followed by Free amino acid (mg/gdw) – 68.0, 109.5, 110.0 and Starch (mg/gdw)- 255.0, 233.3, 112.5. Total soluble sugars in germinating seeds decreased markedly with increasing level of salinity treatment, this is probably due to the fact that under stress condition soluble sugars would provide substrate for respiration to the plant. Starch content during the germination process was decreased with increase in the incubation period irrespective of species and salinity levels. The rate of decrease in starch content during germination process diminished with the increase in salinity levels. This would be possible because under stress conditions starch is hydrolyzed to sugar through enhancement of amylase activity under saline conditions which is further used by plants for respiration and other metabolic processes. It was observed that free amino acids increases as the salinity levels increase. The accumulation of free amino acids might be partially due to hydrolysis /oxidation of proteins in stress conditions.

Conclusions

On the basis of biochemical characterization of different seed varieties of rice, wheat and mustard, it may be concluded that varieties VSR-156, HD-2009, Kranti were salt sensitive, whereas, CSR-10, Kharchia-65, CS-54 were salt tolerant.

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