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DETERMINATION OF TOTAL PROTEIN CONTENT AND SOLUBLE SUGAR IN WHEAT CROP

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ABSTRACT

*A 0.5 ml aliquot was taken in test tube and mixed with 5 ml of reagent (**c**) solution allowed to stand for 10 minutes. Thereafter, 0.5 ml of reagent (**d**) was added with instant mixing. After 30 minutes absorbance was recorded at 570 nm through spectrophotometer (Model SL177) against reagent blank. Standard curve was prepared with a graded concentration of bovine-serum albumin. Similarly the residue left after soluble sugar extraction was suspended with 5 ml of distilled water and 6.5 ml of 52 per cent cold perchloric acid (4^0 C) for 12 hours. It was then centrifuged at 6000-7000 rpm for 20 minutes in a cooling centrifuge at 4^0C. The supernatant was collected in 100 ml volumetric flask. Extraction was repeated with*
5.0 ml of distilled water and 6.5 ml of 52 per cent cold perchloric acid (4^0 C) and centrifuged as above. Both the supernatant were combined and the final volume was made up to 100 ml. 0.5 ml of aliquot was transferred in a test tube and 4.5 ml distilled water was added to it. 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the test tube. Mixed well and placed on a boiling water bath for exactly 8 minutes after which it was cooled to room temperature with ice water. The absorbance was measured at 630 nm against a reagent blank in spectrophotometer. Amount of starch was calculated using standard curve prepared from glucose.

Key words: *reagent, absorbance, concentration, centrifuged.*

INTRODUCTION

When wheat crop is exposed to chronic high temperature range during its grain filling (the period from anthesis to physiological maturity), the size of grain is reduced with increasing temperature and above 32 ^{0}C, premature senescence, inhibition of kernel development and alteration in starch protein composition occurs. The endosperm cells that develop under less than 32 ^{0}C is either smaller or fail to fill out due to reduced starch deposition. Consequently, yield is reduced by 10-15% and in severe condition this is up to 50% (Kamaluddin, 2005).

Increased temperature significantly reduced the days to anthesis, maturity duration and flag leaf area. Likewise, higher post and thesis temperature, i.e. 26.6-30.6 ^{0}C, contributed to decline in productive ears, biological yield, 1000-grain weight and grain yield under late sowing compared with normal sowing. Relative decline in these attributes due to sub-optimal temperature was lower in HD 2279, HI 1116, DW 120, Sonalika and Lok 1, enabling them to perform better under high temperature on late sowing (Singh and Ahmad, 1997).

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REVIEW OF LITERATURE

Yang *et al.* (2002) observed that either stable photosynthesis or high reserve levels provided assimilates for high grain yields in wheat genotypes during heat stress. Combining the two traits could improve heat tolerance of wheat. Viswanathan and Khanna-Chopra (2002) reported that pre-anthesis biomass accumulation rate and grain filing rate were more sensitive to heat stress than duration of phenological phases. Heat tolerant wheat species maintained high stability in thepre-anthesis biomass accumulation rate and grain filling rate.

Studies suggested that decreased conversion of sucrose to starch in the developing wheat endosperm was due to a reduction in the amount of soluble starch synthase activity in the endosperm at elevated temperature (Hawker and Jenner, 1993). Ears of wheat were exposed for upto 10 days during the grain filling stage to high temperature (35 ^{0}C) and activities of five enzymes in sucrose to starch pathway were compared with those ears maintained at lower temperature (21 ^{0}C day/ 16 ^{0}C night). The activity of sucrose synthase and of ADP glucose pyrophosphorylase in ears maintained at 21/16 ^{0}C throughout did not change greatly between days 16 and 32 days after anthesis, whereas, UDP glucose pyrophosphoryase and soluble starch synthase activities declined with advancing development.

Sairam *et al.* (2000) reported that plant protect cell and subcellular system from the cytotoxin effects of the active oxygen radicals using antioxidant enzymes such as superoxide dismutase, ascorbic peroxidase, glutathione reductase, catalase and metabolite like glutathione, ascorbic acid, α-tocopherol and carotenoids. High temperatures reduce the membrane stability of various cellular membranes. Reduction in membrane stability results in loss of physiological function and cell integrity. High temperatures decrease the strength of hydrogen bonds and electrostatic interaction between polar groups of protein within aqueous phase of membrane (Gupta and Gupta, 2005).

MATERIAL AND METHOD

Total protein content (mg g^{-1} fresh weight):

The total soluble protein content was estimated by using method of Lowery *et al.*, (1951).

Principle:

The final colour in the result of biurat reaction of the protein with copper ion in alkali and reduction of phosphomolybdic tungstate reagent by the tyrosine and tryptophan present in the treated protein.

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Reagent:

a) 2 % Na_2CO_3 in 0.1 N NaOH

b) 0.5 % $CuSO_4$. $5H_2O$ in 1 % sodium citrate or sodium potassium tartrate

c) Alkaline copper solution: 1 ml of reagent **b** mixed with 50 ml of reagent **a**.

d) 1N Folin-Ciocalteau reagent (Commercial reagent) diluted with water to give asolution 1 N in acid.

Extraction:

The residues left after 80 % acetone extraction was hydrolyzed in 5 ml of 1 N NaOH for overnight and centrifuged at 5000 rpm for 20 minutes. Supernatant was kept aside and residue was again extracted with 5 ml of 1N NaOH for 1 hr and then centrifuged. Both the supernatants were pooled and made the volume 10 ml.

Procedure:

A 0.5 ml aliquot was taken in test tube and mixed with 5 ml of reagent (**c**) solution allowed to stand for 10 minutes. Thereafter, 0.5 ml of reagent (**d**) was added with instant mixing. After 30 minutes absorbance was recorded at 570 nm through spectrophotometer (Model SL177) against reagent blank. Standard curve was prepared with a graded concentration of bovine-serum albumin.

Determination of total soluble sugar (mg g^{-1} dry weight):

Total soluble sugar was determined according to the method described by Yemm and Willis (1954).

Solution:

(A) Ethanol (80 % V/V)

(B) Anthrone (0.2 % in concentrate H_2SO_4) kept in ice bath

(C) D – glucose

Anthrone reagents:

0.2 g anthrone was dissolved in 100 ml concentrate sulphuric acid.

Procedure:

One hundred mg plant sample was homogenized in 10 ml of 80 per cent ethanol and centrifuged at 4000 rpm for 20 minutes. The supernatant was collected and the residue re- extracted with 10

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ml of 80 per cent ethanol and centrifuged again at 4000 rpm for 20 minutes. Both the supernatant were mixed together. Known amount of ethanol extract (0.1 to 0.2 ml) was evaporated to dryness in a test tube on water bath and cool it to room temperature. One ml of distilled water was added to each test tube and mixed thoroughly. To each test tube 4 ml of anthrone reagent was added along the wall of the test tube and mixed gently, heated on a water bath at 100^0 C for 10 minutes, cooled rapidly under running cold water and absorbance was measured at 620 nm against reagent blank. The amount of total soluble sugar present in the extract was calculated using standard curve prepared from graded concentration of glucose.

Starch determination (mg g^{-1} dry weight):

Starch was estimated through following anthrone reagent method, described by McCready *et al*. (1950).

Anthrone reagent:

0.2 g anthrone is dissolved in 100 ml concentrate sulphuric acid. Store the reagent at 0^0 C but not more than 2 days.

Procedure:

The residue left after soluble sugar extraction was suspended with 5 ml of distilled water and 6.5 ml of 52 per cent cold perchloric acid (4^0 C) for 12 hours. It was then centrifuged at 6000-7000 rpm for 20 minutes in a cooling centrifuge at 4^0C. The supernatant was collected in 100 ml volumetric flask. Extraction was repeated with 5.0 ml of distilled water and 6.5 ml of 52 per cent cold perchloric acid (4^0 C) and centrifuged as above. Both the supernatant were combined and the final volume was made upto 100 ml.

0.5 ml of aliquot was transferred in a test tube and 4.5 ml distilled water was added to it. 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the test tube. Mixed well and placed on a boiling water bath for exactly 8 minutes after which it was cooled to room temperature with ice water. The absorbance was measured at 630 nm against a reagent blank in spectrophotometer. Amount of starch was calculated using standard curve prepared from glucose.

CONCLUSION

Perchloric acid (PCA) hydrolyses glycolytic bonds and produce monosaccharides which are dehydrated to furfural and its derivatives. Furfural reach with anthrone produce blue-green complex. Similarly Sulphuric acid hydrolyse glycosydic bonds of carbohydrate which produces monosaccharides which are dehydrate to furfural and its derivatives. Furfural reacts with anthrone to give a blue-green complex.

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