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Comparative Biochemical Changes in Resistant and Susceptible Cotton Cultivars to Leaf Curl Virus at Germination and Early Seedling Stage: α-amylase, Starch, Total Soluble Sugars in Seed, Radicle and Plumule

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Abstract: Biochemical changes in resistant and susceptible cotton cultivars to leaf curl virus (CLCuV) were assessed by a series of laboratory experiments. The experiments were conducted in petri-dishes containing filter papers with sterile distilled water on which seeds of the cotton cultivars i.e. CIM-446, resistant and S-12 susceptible to CLCuV were sown, each cultivar have five replications and petri-dishes were kept in growth cabinet at 28± 2°C. The results showed that the growth attributes proved S-12 superior to CIM-446. But with respect to the biochemical attributes both the cultivars appear to use different biochemical attributes for their germination demands. Soluble sugars translocations and its concentrations were higher in CIM-446 than S-12. In all the three embryonic organs of germinating seeds, starch mobilization had also the same pattern of varietal difference, where CIM-446 had greater ratio of starch, more active enzyme (α-amylase), degrading starch and higher comparative ratio of the resultant product of starch degradation. This may provide better fulfillment of structural requirements to resist virus at vegetative growth stages by contributing readily available energy by active break down of starch and translocation of sugars which may play some role in the composition of antibodies or some other biochemical/physiological responses associated with starch and sugars.

Key words: Cotton leaf curl virus (CLCuV), physiological/ biochemical changes, α-amylase associated changes

INTRODUCTION

Cotton plant is naturally susceptible to large number of insects and diseases which is due to an organic compound, the gossypol. Among the diseases of cotton, CLCuV is considered to be a serious viral disease[1]. Because it has devastated the economy of Pakistan during the last few years. Cause of the disease is at least two whitefly-transmitted germiniviruses (WTGs) collectively named as cotton leaf curl virus (CLCuV)[2,3]. So the identification of cotton cultivars regarding resistance or susceptibility is necessary at an early growth stages and physiological/biochemical traits or markers may be helpful in this regards. It has been also found that disease resistance or susceptibility depends on physiology of plants differing in their metabolic activities[4]. It is also evident that metabolic activities of enzymes in germinating seeds of cotton vary from cutlivar to cultivar[5]. The work on the enzyme like α-amylase and associated metabolic changes is scantly. As starch is a principal reserve carbohydrate in plants at germination and early seedling growth stages through splitting into sugars. Which in germinating seed provide energy and helps in the formation of other metabolic compounds. It is also observed that in non-photosynthetic tissues as seeds, starch is localized in amyloplasts[6] and is broken down by α and β amylases. When the seed imbibes water there is a rise in α-amylase activity[7-8], which accounts for about 90% of total amylolytic activity[9]. Actually this enzyme is produced interiorly during germination[10] that is why the α-amylase participation in the mobilization of starch is most notable in germinating seeds[11]. Enzymatic breakdown of stored starch to simple sugars and translocation of these sugars to the embryo, where they will provide an energy source for growth[12].

Therefore, these studies were conducted to estimate the Physiological and biochemical basis of resistance and susceptibility to leaf curl virus (CLCuV) in cotton at germination and early seedling stage.

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MATERIALS AND METHODS

Seeds of two varieties of cotton viz., CIM-446 (known CLCuV Resistant) and S-12 (proved CLCuV susceptible) were surface sterilized with 0.1% solution of $HgCl_2$ for 5 min, washed thoroughly in sterile distilled water and placed on filter papers in 14 cm petri-dishes with five replications were kept in a completely randomized design. The seed were allowed to germinate in dark at 28± 2°C in growth cabinets. Fresh samples were collected randomly at every 24 h after sowing for the estimation of fresh weight and for the assay of starch, α-amylase, total soluble sugars. Ten seedlings were homogenized in a mortar and pestle, extracted with cold 1% NaCl in 0.2 M phosphate buffer (pH 5.5), centrifuged at 10,000 g and supernatant used for assy of enzymes according to Chrispeel and Varner[13]. Parallel samples were dried at 65±5 in an oven. Reducing sugars were extracted in 80 % alcohol and were determined according to Riazi *et al.*[14]. Non-reducing and total sugars were determined from the same extract following the method of Riazi *et al.*[14].

RESULTS AND DISCUSSION

Germination percentage in S-12 was significantly higher than that of CIM-446 (Fig. 2 Germination). Dry weight of the cotyledons (Fig.1 Cotyledon) of CIM-446 declined after 2 days imbibition but thereafter it remained unchanged upto day 5 of imbibition. In contrast, dry weight of seeds of S-12 decreased linearly up to day 4. However, dry weights of radicles and plumules [Fig. 1 (radicle), Fig. 1 (Plumule)] of both cultivars increased linearly with time. The most striking and observable change after the onset of germination is growth[7]. Among the growth attributes, the results for germination percentage and dry weights of different embryonic organs evidenced that S-12 was superior to CIM-446. Varietal difference with respect to dry weights of embryonic organs was evident only for cotyledons and plumules, where S-12 and CIM-446 were superior, respectively. Cotyledon dry weights decreased during the same period of time[15]. Similar results were obtained with *Jiangxi lotus*[16]. The decrease in dry weights of cotyledons during

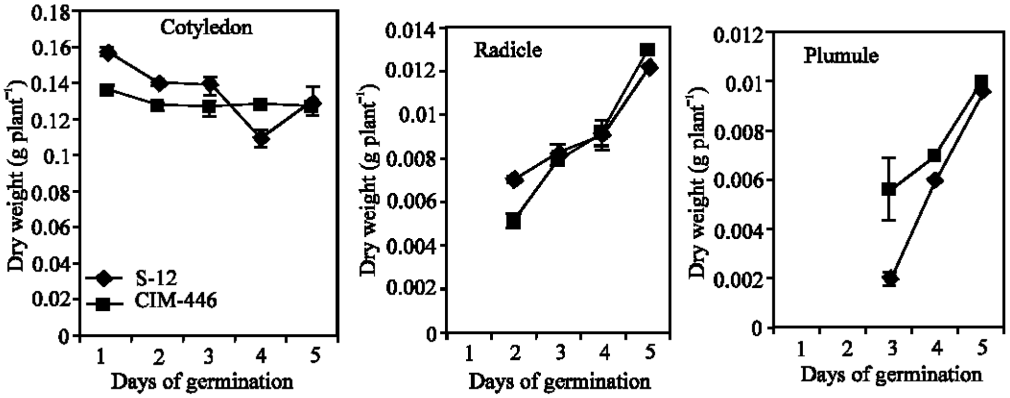

Fig. 1: Time course changes in dry weight per plant in two cotton cultivars differing in CLCuV resistance (Cotyledon, Radicle and Plumule)

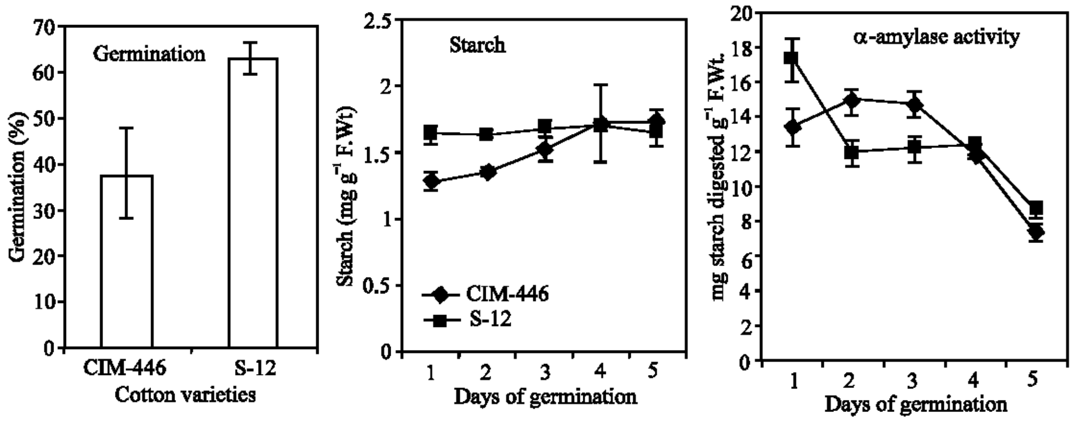

Fig. 2: Time course changes in germination, starch and α-amylase in two cotton cultivars differing in CLCuV resistance

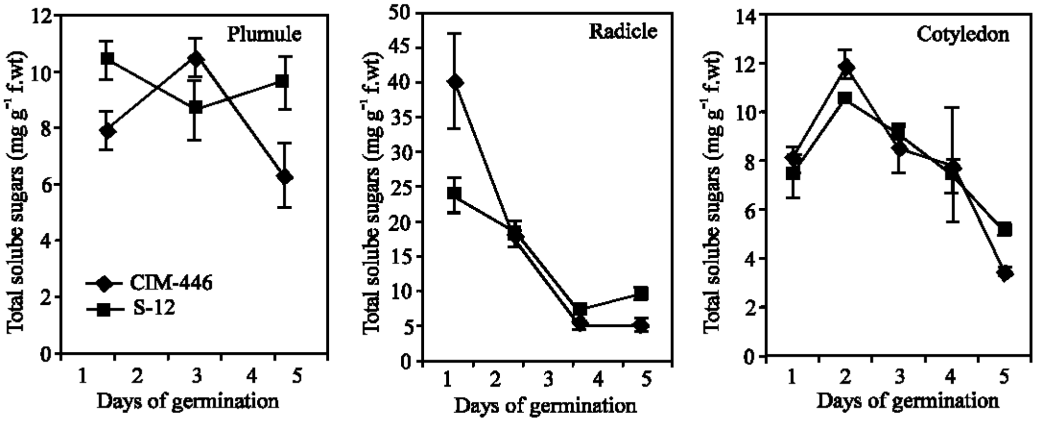

Fig. 3: Time course changes in germination α-amylase, total soluble sugars in (plumule, radicle and cotyledon) of two cotton cultivars differing in CLCuV resistance.

germination with simultaneous increase in radicle and plumule dry weights, is general characteristic of epigeal type of germination.

Starch content in cotyledons (Fig. 2 starch) of both cultivars remained unchanged throughout the entire period of imbibition and the varietal difference with respect to starch content was significant, S-12 being the superior one. α-amylase activity (Fig. 2 α-amylase) in cotyledons of both the cultivars decreased linearly with time of imbibition. However, the varietal difference with respect to α-amylase activity was not possible to discern.

Total soluble sugars in cotyledons (Fig. 3 Cotyledon) first increased up to day 2 of imbibition in both cultivars and then there was a decrease in trend in seed soluble sugars in both cultivars upto day 5 of imbibition. Total sugars in radicles (Fig. 3 Radicle) declined sharply in both the cultivars upto day 5 of imbibition and varieties differed significantly. The varieties had a contrasting pattern for total soluble sugars since in S-12 total soluble sugars in plumules (Fig. 3 Pumule) remained almost unchanged throughout the entire period of imbibition. But in contrast, in CIM-446 there was first an increase in plumule total soluble sugars and then there was a sharp decrease.

Starch is typically broken down by α-amylase and β-amylases. Dry seed contains mainly β-amylases, when the seed imbibes water, there is a rise in α-amylase activity[5,17,18]. In present study cotyledons of CIM-446 confirm these results until first three days of imbibition. Thereafter there was consistent decrease in α-amylase activity that was in agreement with the observations of Prasad *et al.*[17]. In contrast to the pattern followed by CIM-446, a progressive decrease in starch content was observed throughout in S-12 and results were confirmed by Mahanta and Sarma[19].

As far as the cultivars are concerned for biochemical changes associated with the activity of α-amylase both cultivars follow almost the same pattern of time course changes without any notable difference among the cultivars, except for starch content of cotyledons, where cultivars differ significantly, S-12 being superior to CIM-446. Both the cultivars appeared to have used different physiological and biochemical strategies during germination. Higher fresh weights and moisture contents in the embryonic organs of S-12 may be attributed to higher germination percentage of S-12 as compared to CIM-446. The higher germination of S-12 seemed to be determined by higher starch. However, there is a need to elucidate whether the biochemical differences observed between the two cultivars during seed germination are maintained at the later growth stages and how far these biochemical attributes play a role in resistance to CLCuV.

REFERENCES

1. Muhammad, F., A.H. Tariq, J. Ihsan and A. Saleem, 1998. Evaluation of two cotton leaf curl virus transmission techniques and their response to different cotton cultivars. Pak. J. Phytopathol., 10: 18-22.
2. Hameed, S., S. Khalid, E.U. Haq and A.A. Hashmi, 1994. Cotton leaf curl disease in Pakistan caused by a whitefly transmitted geminivirus. Plant Dis., 78-80.
3. Harrison, B.D., Y.L. Liu, S. Khalid, S. Hameed, G.W. Otim-Nap and D.J. Robinson, 1997. Detection and relationships of cotton leaf curl virus and allied whitefly-transmitted geminiviruses occurring in Pakistan. Ann. Appl. Biol., 130: 61-75.

4. Dawson, W.O. and M.F. Hilf, 1992. Host-range determinations of plant viruses. Annu. Rev. Plant Physiol. Plant Mol. Biol., 43: 527-555.
5. Gupta, M.P., 1991. Amylase activity in cotton. Indian. J. Agric. Res., 25: 141-148.
6. Dey, P.M. and J.B. Harborne, 1997. Plant Biochemistry. Academic Press, pp: 177-184.
7. Mayer, A.M. and A. Poljakoff-Mayber, 1989 The Germination of Seeds. 4th Ed. Pergamon Press. Oxford, pp: 1-194.
8. Kohno, A. and T. Nanmori, 1991. Changes in α-amylase and β-amylase activities during germination of seeds of alfalfa (*Medicago sativa* L.). Plant Cell Physiol., 32: 459-466.
9. Ashraf, M.Y., G. Sarwar, M. Ashraf, R. Afaf and A. Sattar, 2002. Salinity induced changes in α-amylase activity during germination and early cotton seedling growth. Biologia Plantarum, 45: 589-591.
10. Thomas, B.R. and R.L. Rodriguez, 1994. Plant Physiol., 106: 1235-1239.
11. Minamikawa, T., D. Yamanuchi, S. Wade and H. Takeuchi, 1992. Plant Cell Physiol., 33: 253-258.
12. Devlin, R.M. and F.H. Witham, 1969. Plant Physiology. 4th Edn. Wadsworth Publishing Company Belmont, California, pp: 400.
13. Chrisspeels, M.J. and K.E. Varner, 1967. Gibberrelic acid enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol., 42: 398-406.
14. Riazi, A., K. Matruza and A. Arslam, 1985. Water stress induce changes in concentration of proline and other solutes in growing regions. J. Expt. Bot., 36: 1716-1725.
15. Prisco, J.T., I.L. Ainouz and D.E.C. Melo, 1974. Changes in nitrogenous compounds and proteases during germination of *Vigna sinensis* seeds. Physiol. Plant, 33: 18-21.
16. Maeda. Y., T. Minamikawa and B.M. Xu, 1996. Metabolic activities in germinated ancient lotus seeds. J. Exp. Bot., 297: 577-582.
17. Prasad, J.S., M. Mishra, R. Kumar, A.K. Singh and U.S. Prasad, 1996. Enzymatic degradation of starch in germinating litchi (*Litchi chinensis* Sonn.) seeds. Seed Res., 22: 89-97.
18. Kapoor, R., 1998. Biochemical changes during imbibition and germination of safflower seeds (*Carthamus tinctorius* var. Bhima). J. Physiol. Res., 11: 29-34.
19. Mahanta, P.K. and C.M. Sarma, 1996. Mobilization of cotyledon reserve during early seedling growth of tea (*Camellia sinensis* (L.) O. Kuntze) in dark and light-interrupted dark. Seed Res., 23: 1-4.