Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 06, Issue, 08, pp. 9064-9068, August, 2016

Full Length Research Article

BIOCHEMICAL SCREENING OF ADVANCED POTATO LINES FOR TOLERANCE AGAINST *RHIZOCTONIA SOLANI*

*[1]Habib Ullah, [1]Sahib Alam, [1]Hamid Ullah Shah [5]Misbah Ullah, [2]Alam Zeb, [3]Sohail Kamaran, [2]Gul Roz Khan and [4]Rehmat Gul

[1]Department of Agricultural Chemistry, University of Agriculture Peshawar, Pakistan
[2]Department of Agronomy, University of Agriculture Peshawar, Pakistan
[3]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
[4]Department of Human Nutrition, University of Agriculture Peshawar, Pakistan
[5]Department of Entomology, University of Agriculture Peshawar, Pakistan

ARTICLE INFO

Article History:
Received 11th May, 2016
Received in revised form
22nd June, 2016
Accepted 13th July, 2016
Published online 30th August, 2016

Key Words:
Potato,
Rhizoctonia solani,
Reducing sugar,
Starch and vitamin C.

ABSTRACT

Potato (S*olanium tuberosum*) is a starch rich tuberous crop of Solanaceae family. Potato is known to be indigenous to Chiloe Archipelago and its cultivation dates back 10,000 years. In the early and mid sixteenth century it was introduced to Europe and the marines introduced it to the territories all over the world. In Pakistan potato was cultivated on an area of 154317 hectares with total production of 2538971 tons in the year 2007-08. During the present study three potato lines viz. VR96, VR94725 and V3 were investigated for reducing sugar, starch and vitamin C after artificial inoculation with *Rhizoctonia solani* for 40 days and compared with control. The reducing sugar content in control samples ranged from 13.22 to 13.38% while in inoculated samples it ranged from 5.51 to 5.22%. Starch and vitamin C content were significantly affected by inoculation. In control the starch varied from 38.37% in VR96 to 46.56% in V3 whereas in inoculated samples the starch ranged from 24.27 % in V3 to 31.09% in VR94725. Similarly the vitamin C ranged from 3.32 to 4.25mg/100gm in control samples whereas in inoculated samples it ranged from 1.69 to 2.21mg/100gm.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual, herbaceous and dicotyledonous plant belonging to genus *Solanum* and family Solanaceae, a major vegetable crop of Pakistan, grown on an area of 185.1 thousand hectares with an annual production of 4104.4 thousand tonnes (Anonymous, 2013). Potato belongs to one botanical specie *Solanum tuberosum* but it comprises of thousands of varieties that vary in their characteristics. Potato's origin is from South America Andes. Chile and Peru fight over its origin (Anonymous, 2008). Haq *et al*. (2008) reported that half of population of Pakistan is at threshold level of food insecurity because of shortage and surge in prices of food items according to World Food Program. Researchers at International Potato Center have recommended that the emergent dilemma of food supply due to rise in prices and production of crops intended for bio-fuel rather than foodstuff could be lessened by an increase in potato cultivation (CIP, 2008). Potato is known to be the richest of carbohydrates with 26 g within a medium potato. Small amount of this starch is resistant to be digested by the enzyme in stomach and small intestine, which reaches the large intestine having the same physiological effect on the body like fibers that provide bulk, protects the body from colon cancer, and increases glucose tolerance and insulin sensitivity, lower the cholesterol level and triglyceride concentration and also reduces the fat storage (Raban *et al*, 1994; Hylla *et al*, 1998). Studies on various potato varieties from Punjab region contained moisture up to 80 %, dry matter 23 %, ash 6 %, crude protein 14 %, crude fat 1 % and starch 76 % (Bhatti *et al*., 1968). The resistant amount of starch in potato depends mostly on preparation method to be used. This resistant starch increases on cooking and then by cooling. For example, the amount of resistant starch in cooked potato is about 7 %, which increases to 13% upon cooling. This contains toxic compounds such as glycolalkaloides, the

Corresponding author:* ***Habib Ullah,*
Department of Agricultural Chemistry, University of Agriculture Peshawar, Pakistan

most prevalent of which is solaline and chaconine. These compounds are present mostly in sprouts, leaves and stems which protect plants from predators. Glycoalkaloides causes headache, and sometimes leads to coma and even death in severe cases. In humans the poisoning occurs very rarely (Englyst *et al*., 1992). Potato crop is having serious and major pathological threats in Pakistan and during recent years, soil-borne and seed-borne diseases have turned out to be a major threat to this crop in Pakistan (Ahmad *et al*., 1995).

Among the various fungal diseases of potato crop, black scurf disease of potato caused by, Rhizoctonia solani, (Ahmed *et al*., 1995; Khan *et al*., 1995) is the serious and most commonly observed disease with the characteristic symptoms of black scurf (dark brown to black colored hard masses of sclerotia, irregularly shaped and superficial, varying from small, flat, barely detectable blotches to large and raised lumps adhering tightly to the skin) on tubers and stem canker are the result of *Rhizoctonia* disease complex in potato (Tsror, 2010). Black scurf disease increases gradually as level of inoculum increases and sclerotia may develop on tuber surface even under primary low inoculum level, resultantly the control through fungicidal chemistries is not always useful especially when levels of initial inoculum are high (Tsror and Peretz 2005; Kyritsis and Wale, 2002). Rhizoctonia solani Kühn, (teleomorph Thanatephorus cucumeris (A. B. Frank) Donk) is a soil borne Basidiomycete occurring world-wide, with complex biology and causes disease on a broad array of host plant species (Agrios 1988). Keeping in view the importance of potato in human nutrition as well as the economy of country, this study was planned to investigate and compare the proximate composition, reducing sugars, starch, minerals and vitamin C contents of *Rhizoctonia solani* infected three newly developed potato lines.

MATERIALS AND METHODS

Samples collection

Composite samples (2 kg) each of the three newly developed potato lines (VR96, VR94725 and V3) were collected from Agricultural Institute Tarnab (ARI Tarnab), Peshawar. The samples were brought to the post graduate laboratory of Department of Agricultural Chemistry, Khyber Pakhtunkhwa, University of Agriculture Peshawar, Pakistan where they were reduced to laboratory samples (1 kg each) and stored at 4 ^{0}C for further study.

Inoculation with *Rhizoctonia solani* and storage

Rhizoctonia solani mycelia were isolated from the naturally infected potato tubers. For this purpose, an inoculum was made from the infected portions of potato tuber and was spread plated on potato dextrose agar held in Petri plates. After incubation for seven days, at 28 ^{0}C, mycelia from *R. solani* colonies were isolated and purified by sub-culturing on Malt Extract Agar (Shah *et al*., 2010). From the purified isolates of *R. solani* mycelial suspensions were made in distilled water. The samples of selected potato lines were taken in plastic jars and inoculated with *R. solan*i suspension. Three replicates per control (uninoculated) and inoculated samples of each line were taken and incubated at ambient room temperature. A total of 18 replicates were incubated for 40 days. At the end of experimental period all the samples were analyzed and compared for the following parameters.

Reducing Sugar

For the calculation of reducing sugar the method of AOAC (2000) was followed. 2 g of the potato sample was extracted with 100 ml distilled water by crushing and subsequent filtration. The filtrate was taken in burette. Then 5 ml of Fehling A (7 g $CuSO_4.5H_2O$ per 100 ml water) and 5 ml of Fehling B (10 g NaOH plus 34 g sodium potassium tartarate per 100 ml distilled water) solutions along with 10 ml of distilled water were taken in a conical flask. The flask was heated continuously and the sample solution was added from the burette drop by drop until break red color was observed. Then few drops of methylene blue was added as indicator which did not change the color from red to blue and reduction was completed. The amount of reducing sugar was calculated by the fact that 5 ml of Fehling A + 5 ml of Fehling B reduce 0.05 g of the reducing sugar. Reducing sugar (%) was calculated by using the following formula:

$$reducing\ sugar\ (\%) = \frac{0.05 \times volume}{titration\ reading}$$

Starch determination

Starch was determined by the method as reported by Potzi (2006). Accurately weighed 8 g of sample taken on a filter paper was washed twice with 10 % alcohol and then with 30 % alcohol (20 ml each). The residue was transferred into beaker containing 50 ml of water and heated for 15 minutes with constant stirring until the starch was gelatinized and homogenous mixture was observed. The solution was cooled to 50^{0}C and was added with 0.03 g of diastase enzyme dissolved in 5 ml of water. The mixture was then kept at 60^{0}C in incubator for one and half an hour. The temperature was raised to 100 ^{o}C and then filtered. The residues were then hydrolyzed with 0.1 N HCl and heated for 2 hours. The mixture was cooled and neutralized with 0.1N Na_2CO_3 and the volume was made upto 500 ml. The calculation of dextrose was carried out by Lane and Eynon method (1923). For this purpose, 5 ml of Fehling A and 5 ml of Fehling B were pipetted into a conical flask boiling on asbestos gauze and added with the test solution till the blue color was disappeared. 2 ml of methylene blue was added and the process was continued until blue color was disappeared completely. The end point was brick red color.

Amount of starch = dextrose x 0.90

$$\%\ Starch = \frac{Weight\ of\ starch}{Weight\ of\ sample} \times 100$$

Vitamin C determination

Dye reduction method was employed for calculation of Vitamin C. The ascorbic acid present in sample was titrated against 2, 6-diclorophenole indophenol as suggested by AOAC (2003).

Reagents

- 50 mg of dye (2, 6-dichlorophenol indophenol) and 42 mg $NaHCO_3$ dissolved in 200 ml boiled distilled water homogenized for 30 minutes.
- Oxalic acid 0.4 %
- Standard ascorbic acid (50 mg ascorbic acid dissolved in 50 ml of 0.4 % oxalic acid)

Standardization of Dye

Oxalic acid and ascorbic acid in ratio of 1:25 ml were taken in the conical flask and titrated against dye. The dye factor was calculated as follow.

Dye factor = 1/ titration reading

Sample assay

20 ml of sample and 50 ml of 0.4 % oxalic acid were taken and homogenized. The volume was then made up to 500 ml with oxalic acid solution. 10 ml of this solution was titrated against dye resulted in a pink color.

$$\% \text{ Vitamin C} = \frac{\text{Titration reading x dye factor x dilution factor}}{\text{Volume of sample x aliquot sample}} \times 100$$

Statistical analysis

The data was subjected to statistical analysis using MSTATC package of statistical software. Analysis of variance was carried out by using CR design. Means were compared by Least Significant Difference (LSD) test at P≤0.05.

RESULTS AND DISCUSSION

A comparative study was carried out to check the nutritional stability of newly developed potato lines (VR96, VR94725 and V3) against *Rhizoctonia solani*. For this purpose, the samples of the selected lines were artificially inoculated with *R. solani* mycelia. After 40 days of storage all the samples were examined for proximate composition, reducing sugar, starch, vitamin C content and minerals profile were compared with control. The data so obtained were presented as tabulated and graphically and is discussed as follows:

Reducing sugar

Data concerning reducing sugar content are shown in Figure 1. It was examined that reducing sugar ranged from 13.22 to 13.38% in control samples. Higher reducing sugar content was found in V3 and lower was recorded in VR94725. In inoculated samples, 5.51 % was the higher and 5.22 % was the lower sugar content in V3 and VR96 respectively. Analysis of data revealed that control and inoculated samples were significant (P≤0.05) while their interactions were not significant with respect to reducing sugar contents. Similar results have been reported in earlier literature that the reducing sugar contents of plants are decreased with the attack of microorganisms. Killadar and More (2010) observed a decrease in reducing sugar content of *Memcylon umbellatum* from 23 to 16 % as infected by fungi.

Starch content (%)

Figure 2 represents the data regarding the starch content of selected potato lines. It was observed that, among the control (uninoculated) treatments V3 had the highest amount of starch (46.56 %), followed by VR94725 (42.02 %) and the minimum amount (38.37 %) was examined in VR96. Starch content of all the three lines were negatively affected by *Rhizoctonia solani.* In inoculated samples, the average starch contents were found to be 26.26 %, 31.09 % and 24.27 % for V3, VR94725 and VR96, respectively. Our findings are fairly in line with that of Amadioha (1998) who reported that starch content was decreased from 70 to 17.7% in *Rhizopus oryzae* infected potato cultivars during 10 days storage. Similar pattern of results were reported by Mba and Akueshi, (2001) and Ataga and Ota, (2006).

The decrease in starch content during infection may be explained by the fact that nutrient components generally decrease where there is a disease pressure. When the plants are attacked by external pathogens they show a decrease in the nutrient composition to minimize the disease pressure (Shehu and Aliero, 2010). Potato is a best source of starch. A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber: It provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage (Raben *et al*., 1994; Cummings *et al*., 1996; Hylla *et al*., 1998).

Table 1. Analysis of variance for reducing sugar content, starch content and Vitamin C content of potato lines

SOV	Sugar	Starch	Vitamin C
Factor A	4727812.50**	1028.00**	12.24**
Factor B	5.17**	28.84**	0.10NS
AB	58.50*	58.55**	0.93**
Error	3600.39	2.05	0.06
CV	9.22	4.12	8.32

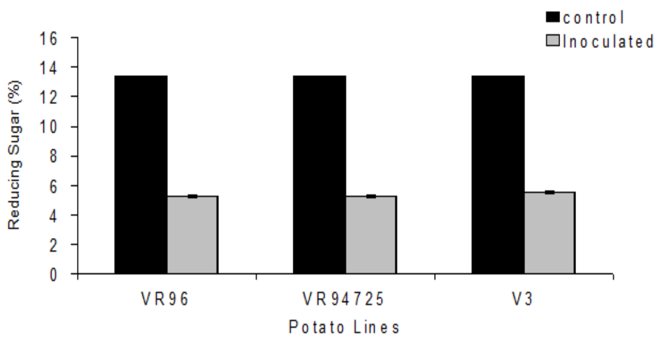

Fig.1. Reducing Sugar content (%) of three potato lines infected by *Rhizoctonia solani*. Vertical bars represent the standard errors of means

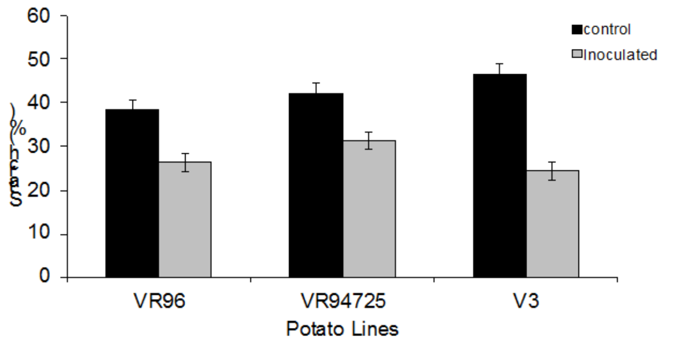

Fig.2. Starch content (%) of three potato lines infected by *Rhizoctonia solani.* Vertical bars represent the standard errors of means

Vitamin C content

The data pertaining to vitamin C contents of the newly developed potato lines (i.e. VR96, VR94725 and V3) as affected by *Rhizoctonia solani* is shown in figure 3. It was observed that in control samples, maximum vitamin C (4.27 mg/100g) was found in VR96 whereas minimum (3.31mg/100g) in VR94725. Inoculation significantly reduced the vitamin C content of all the three lines. Vitamin C contents of inoculated samples were found to be 1.69, 2.11 and 2.21 mg/100g for VR96, VR94725 and VR96, respectively. Analysis of the data showed that VR96 was most susceptible to *Rhizoctonia solani* in term of vitamin C reduction. These results are strongly supported by the finding of (Aulakh *et al.,* 1970; Farooqui *et al.,* 1080), who reported that the concentrations of vitamin C are adversely affected by different plant pathogens. They reported the change in ascorbic acid content in crops as results of fungal and nematode infection. Ascorbic acid is water-soluble vitamin. High amount required for body, as its excretion is frequent from body. It plays an important role in reversible oxidation-reduction system. It works against scurvy disease and also helps in the formation of folic acid derivatives and essential for DNA synthesis (Chatterjea and Shinde, 1998).

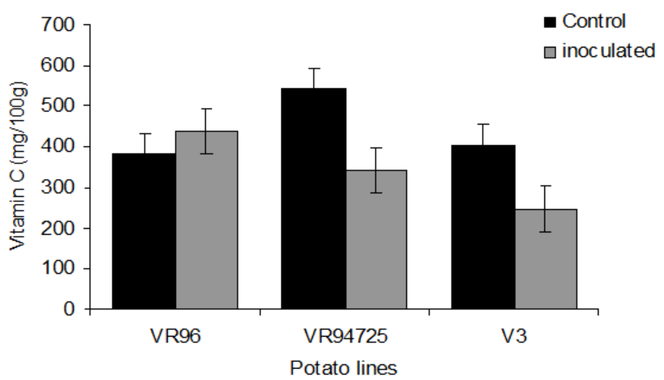

Fig. 3. Vitamin C content (mg /100 gm) of three potato lines as infected by *Rhizoctoni solani.* Vertical bars represent the standard errors of means

Conclusion

It is concluded that the reducing sugar, starch and vitamin C in potato lines (VR96, VR94725 and V3) were significantly decreased with *R. solani* infection. Hence it is concluded that inoculation significantly affected the nutritional quality of almost all potato lines but VR-96 was less affected.

REFERENCES

A.O.A.C. (Association of Official Analytical Chemists). 2000. Official Methods of Analysis, (15th ed.).

Ahmad, I., Soomro, M. H., Khalid, S., Iftikhar, S., Munir, A., & Burney, K. 1995. Recent distributional trends of potato diseases in Pakistan. National Seminar on Research and Development of Potato Production in Pakistan, April 23-25, NARC, PSPDP, PARC, Islamabad, Pakistan.

Amadioha. 1998. Effect of infection by *Rhizopus oryzae* on biochemical composition of stored potato tubers. Plant Foods for Human Nutr. 53: 145-151.

Anonymous. 2008. Agricultural Statistics of Pakistan. Govt. of Pakistan. Ministry of Food, Agriculture, and Livestock. Food, Agriculture & Livestock Division (Economic Wing), Islamabad.

Anonymous. 2013. Agricultural Statistics of Pakistan. Govt. of Pakistan. Ministry of Food, Agriculture, and Livestock. Food, Agriculture & Livestock Division (Economic Wing), Islamabad.Washington DC.

Ataga, E.A., and Ota. 2006. Seed-borne fungi of the wild mango and their effects on food composition. *Nig. J. Bot.* 19: 54-60.

Aulakh, K.S., R.K. Grover and S. Malhotras. 1970. Change in vitamin C and carbohydrate contents of tomato fruits inoculated with isolates of Puma destructiva Plow. Phytopath. Medit. 9: 91-94.

Bhatti, M.B., M.I. Siddiqi and S.M. Umar. 1968. Certain dehydration studies on potatoes. In Composition of varieties. *J. Springer.* 19: 17-33.

Chatterjea, M. N., and R. Shinde.1998. Text book of Medical Biochemistry. Jaypee Brother Medical Publishers: pp.173

CIP. 2008. Potatoes and the environment. Fact sheet. CIP, Lima, Peru.

Cummings, J.H., E.R. Beatty, S.M. Kingman, S.A. Bingham, and H.N. Englyst. 1996. Digestion and physiological properties of resistant starch in the human large bowel. Br. *J. Nutr.* 75: 733-747.

Englyst, H.N., S.M. Kingman and J.H. Cummings. 1992. Classification and measurement of nutritionally important starch fraction. *Eur. J. Clin. Nutr*. 46: 33-50.

Farooqui, T. N. A., A.K. Gangulay, and D.R. Gupta. 1980. Some chemical changes in tomato plants infected with root- knot nematode. *Indian. J. Ent*. 42: 226-232.

Haq, Z., H. Nazli and K. Meilke. 2008. Implications of high food prices for poverty in Pakistan. Agricultural Economics, 39, 477-484.

Hylla, S., A. Gostner, G. Dusel, H. Anger, H.P. Bartram, S.U. Christl, H. Kasper, and W. Scheppach. 1998. Effects of resistant starch on colon in healthy volunteers: possible implications for cancer prevention. *Am. J. Clin. Nutr.* 67:136-142.

Khan, R. A., S. Iftikhar, A. Rafi, S. Riaz, and I. Ahmad. 1995. Distribution and incidence of tuber diseases of potato in Swat valley. National Seminar on Research and Development of Potato Production in Pakistan, April 23-25. 1995, NARC, PSPDP, PARC, Islamabad.

Killedar, S.G., and H.N. More. 2009. Analgesic and anti-inflammatory studies of *Memocylon umbellaturm* Burm roots. *J. Pharm.Tech*. 2:858-861.

Kyritsis, P., and S. J. Wale. 2002. Effect of mycelial inoculum level and cultivar susceptibility on Rhizoctonia solani development on potato stems and seed tubers. The-BCPC Conference Pests and disease (Volumes 1and 2, pp. 761-764). Proceedings of an international conference held at Brighton Hilton Metropole Hotel Brighton UL, 18-21 November 2002.

Mba, M.C., and C.O. Akueshi. 2001. Some physico-chemical changes induced by *Aspergillus flavus* and *A. niger* on *Sesanum indicum* and *S. radiatum*. *Afri. J. Natural Sci*. 4: 94-97.

Potz J., 2006. Determination of potato starch content in slurry by an aerometer. 44: 283-284.

Raban, A., A. Tagliabue, N.J. Christensen, J. Madsen., J.J. Host and A. Astrup. 1994. Resistant starch: the effect on postprandial glycemia, hormonal response, and satiety. *Am. J. Cli. Nutr*. 600:544-551.

Shah, H.U., T.J. Simpson, S. Alam, K.F. Khattak, S. Perveen. 2010. Mould incidence and mycotoxin contamination in maize kernels from Swat Valley, North West Frontier Province of Pakistan. *Food Chem.Toxicol*. 48: 1111–1116.

Shehu, K and A.A. Aliero. 2010. Effects of purple Blotch infection on the proximate and mineral contents of onion leaf. Inter. J. *Pharma Sci. Res*. 1:131-133.

Solis, J.S., 2007. Molecular description and similarity relationships among native germplasm potatoes (Solanum tuberosum ssp. tuberosum L.) using morphological data and AFLP markers. *Elec. J. Biotech*. 10:432-436.

Thesen, K., 2007. World potato Atlas: China-History and overview. Inter. Potato Centr. Notes. 45: 477 532.

Tsror, L. 2010. Biology, Epidemiology and Management of *Rhizoctonia solani* on Potato. *Journal of Phytopathology*, 158, 649-658

Tsror, L., and I. peretz- Alon.2005. The influence of the inoculums source of Rhizoctonia Solani on development of black scurf on potato. *J. Phytopath. 153*: 240-244

****