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ALLELOPATHIC ASSESSMENT OF GENETICALLY MODIFIED AND NON MODIFIED MAIZE (*ZEA MAYS* L.) ON PHYSIOLOGY OF WHEAT (*TRITICUM AESTIVUM* L.)

MUHAMMAD IBRAHIM[1], NASEER AHMAD[1], ZABTA KHAN SHINWARI[2*] ASGHARI BANO[1] AND FAIZAN ULLAH[1]

[1] *Department of Plant sciences Quaid-i-Azam university Islamabad Pakistan*
[2]*Department of Biotechnology Quaid-i-Azam university Islamabad Pakistan*
[*]*Corresponding author, s e-mail: Shinwari@qau.edu.pk*

Abstract

The aim of this work was to study the allelopathic effect of 3 extracts viz 3%,5% and 10% prepared from leaves of GM (insect resistance) and non GM maize on physiology of succeeding crop wheat (*Triticum aestivum* L.). The extracts prepared from GM maize significantly decreased chlorophyll a content but significantly increased chlorophyll b content as compared with untreated control. Content of chlorophyll a non-significantly decreased but content of chlorophyll b significantly increased with methanolic extract prepared from non GM maize. Significant decrease in carotenoid content was found with aqueous extracts of both GM and non GMmaize.GM maize leaves extracts significantly decreased proline but have no effect on protein and sugar content of wheat plant. Significant increase in protein and sugar content was found with aqueous extract of non GM maize as compared with untreated control. Significant increase in superoxide dismutase (SOD) and non-significant increase in catalase was found with aqueous extracts however activity of peroxidase (POD) decreased in wheat crop with GM maize leaves extracts. Non-significant increase in antioxidant activities were found with treatment of non GM maize leaves extracts as compared with untreated control.

Introduction

There is increasing emphasis on sustainable agriculture and concerns about the adverse effects of extensive use of synthetic chemicals, such as contamination of the environment, greater plant resistant to herbicides and high costs. As a result, research attention is now focused on decreasing the dependence on the synthetic herbicides and finding alternative strategies for weed management and insect control. In agro-ecosystem allelopathy is one of promising strategy, which can be put into good use in several ways (Khan *et al*., 2009).

Plants have been engineered to have several required characters, such as insecticide resistance to pests, or tolerate to stressful environmental conditions, improved nutritional value. Since the first commercial cultivation of genetically modified plants in 1996, that has been modified to be tolerant to the herbicides glufosinate and glyphosate, to be resistant to virus damage as in ring spot virus resistant. As we know the world population is increasing day by day reached to over 7 billion people and is expected to double in the coming 50 years (Anon., 2011). In the near future guaranteeing a sufficient food supply for this increasing population is going to be a big challenge. In many ways only GM foods guarantee to convene this requirement. Throughout the world during 20th century there has been a great deal of development in the field of biotechnology and rapid development of biotechnology has promoted the research and acceptance of genetically modified (GM) crops in many countries. The significant achievements in transgenic biotechnology has noticeable impact on the world crop manufacture and crop growing forms of agricultural species such as cotton, soybean, canola, and maize (Shinwari *et al*., 2010). With global cultivation of genetically modified (GM) crops having reached 134 million hectares and covering 25 countries, a new gesture of adoption of biotech crops is causal to a broad based and continuing hectarage growth worldwide (James, 2010).The state with the largest area of GM crops (half of the world's total acreage) is the United States of America (USA), followed by Argentina, Brazil, Canada, India and China The global acreage of GM crops reached a record 125 million hectares (309 million acres) in 25 countries in 2009 (James, 2010).

There are 2 types of genetically modified maize grown all around the world (a) herbicide tolerance e.g. Monsanto's roundup ready (b) insect resistance e.g. Monsanto's MON 810, Pioneers 1507 and Syngenta's Bt11. Through the insertion of a gene from soil bacterium *Bacillus thuringiensis* (Bt) MON 810, Bt11 and 1507 maize has been genetically modified to create a pesticide, the Bt protein or toxin. Due to agricultural practice and after harvesting of genetically modified crops, in many countries of the world how much amount of Cry1Ab remains in the soil is questionable. It is reported by Einspanier *et al*., (2004) that high level of toxin is found in the soil close to the roots and remaining plant residues. In Pakistan maize production has been increased from 0.38 million tons during 1947-50 to 3.037 million tons in 2007. The area of cultivation was 935.1 million ha and production was 3261.5 million tons in 2009-1010 (Anon, 2010), grading third after wheat and rice (9131.6 and 2883.1 million ha). Due to its importance, maize was one of the first crops to be genetically engineered and commercially released but due to biosafety issues transgenic maize is not commercially grown in Pakistan. Wheat (*Triticum aestivum* L.) is important staple food of Pakistan and wheat straw is an essential part of the daily ration of live stocks in majority areas of our country. Various factors lower the productivity of wheat such as delayed sowing, water shortage, disease and drought. Keeping in view the biosafety issues of transgenic maize, the present research work was conducted to evaluate the allelopathic effect of genetically modified and non-modified maize leaves extracts on physiological aspect of wheat crop.

Materials and Methods

The research was carried out in the field area of Institute of Agri-Biotecnology and Genetic Resources (IABGR), National Agricultural Research Centre, Islamabad, Pakistan (NARC) and Department of Plant Sciences, Quaid-i-Azam University, Islamabad Pakistan in randomized complete block design with 3 replications under control conditions. Seeds of genetically modified maize (Insect resistance), non modified maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) cv. Chakwal-97 were obtained from IABGR Gene bank, National Agricultural Research Centre Islamabad Pakistan.

Preparation of plant extracts: Leaves were cut into small pieces less than 2cm and were shade dried under normal conditions. The dry plant materials were utilized for preparation of methanolic and aqueous extracts. Extracts from leaves of both GM and non GM maize were separately prepared. Plant leaves (10g) were added into 100 ml methanol (1:10 w/v) and kept in shaker for 1hour. After shaking for an hour, extracts were placed at room temperature for 48 hours following method of Wardle *et al*., (1992). The extracts were then filtered with muslin cloth followed by Whatman filter paper No.1. The filtrates were dried by rotary evaporator and used for different fraction preparation.

Seed surface sterilization: The seeds of succeeding crop wheat (*Triticum aestivum* L.) cv. Chakwal-97 were surface sterilized with 95% ethanol and 10% chlorax for 5 minutes and thoroughly washed with sterile water several times. Stock solution (10%) was further diluted to 3% and 5% and 10% solutions respectively. Methanolic and aqueous extracts (10ml) were poured to each small sized beaker and then 30 seeds were socked for 8 hours in each beaker. Sterile water was used as a control. Twenty seeds based on treatments were sown evenly in earthen pots (23 × 24 cm) filled with clay and sand in ratio of 7:1 and kept under natural conditions during month of November, 2010. Wheat plants were harvested with fully expanded green leaves at vegetative stage after 40 days after sowing and samples were freeze dried for physiological, biochemical analysis. The following traits were taken into account.

Chlorophyll (a, b) and carotenoid contents of leaves: Chlorophyll a, b and carotenoid contents of fresh leaves of wheat were determined by the method of Arnon (1968).

Leaf protein contents: Protein content of fresh leaves of wheat was determined following the method of (Lowery *et al*., 1951) using Bovine Serum Albumin (BSA) as standard.

Proline contents of leaves: Proline contents of leaves were determined followed the Bates *et al*., (1973) protocol.

Sugar estimation: Sugar estimation of fresh leaves was determined by using Dubois *et al*., (1956) method.

Assays for activity of superoxide dismutase (SOD): SOD activity was determined by measuring inhibition of photochemical reduction of nitrobluetetrazolium (NBT) using method of Beauchamp and Fridovich (1971).

Assay for peroxidaze activity (POD): POD activity was determined by the method of Vetter *et al*., (1958) as modified by Gorin and Heidema (1976). The assay mixture contained 0.1 ml enzyme extract, 1.35ml 100mM MES (Methyl ethane sulphate) buffer (pH 5.5), 0.05% H_2O_2 and 0.1% p-phenylenediamine. Changes in absorbance were recorded at 485nm for 3 minutes with the spectrophotometer. The activity of POD was presented as OD485 nm /min /mg protein.

Assays for catalase activity (CAT): Catalase activity (CAT) was measured according to Chandlee and Scandalios (1984). The assay mixture contained 2.6ml of 50mM potassium phosphate buffer (pH 7.0), 0.4ml of 15mM H_2O_2 and 0.04ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in the absorbance at 240nm. The enzyme activity was expressed in Umg^{-1} protein (U = 1mM of H_2O_2 reduction min^{-1} mg^{-1} of protein).

Statistical analyses: The data was analyzed statistically by analysis of variance technique and comparison among treatment means was made by Duncan's Multiple Range Test (DMRT) using Costate version 6.400 (Duncan's, 1961).

Results and Discussion

The experiment was carried out to determine the effect of methanolic and aqueous extracts prepared from leaves of GM (insect resistant) and non-GM maize on growth and physiological attributes of wheat (*Triticum aestivum* L.) cv. Chakwal-97 in pot experiment under natural environmental conditions. Among physiological attributes, the effects of methanolic and aqueous extracts of GM and non-GM maize were determined on photosynthetic pigments, leaf soluble proteins, sugars, stress related amino acid proline and antioxidant enzymes. Chlorophylls and carotenoid are crucial plant pigments for photosynthesis and their abundance results in greater assimilation of solar radiations into consumable sugars. Leaf photosynthetic capacity depends on physiological characteristics such as chlorophyll contents, rubisco activity and photo system efficiency (Bowes, 1991). During present investigation there was found that both GM and non-GM extracts significantly decreased the amount of chlorophyll a. There are several reports that chlorophyll content of leaves decreased under stressful conditions. The results obtained are in agreement with those of Jaleel *et al*., (2008) & Al-Sobhi *et al*., (2006). Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions (Ali *et al*., 2004). Maximum significant increase in chlorophyll b content was found with methanolic extract of GM maize

as compared with untreated control. Chlorophyll b content generally increased with GM maize extracts as compared with non GM one. Carotenoid is responsible for quenching of singlet oxygen (Knox & Dodge, 1985) and thus help in overcoming oxidative stress. Maximum significant decrease in carotenoid content was observed in wheat crop receiving at 5% aqueous extract of GM maize and 10% aqueous extract of non-GM maize as compared with untreated control. The decrease in carotenoid contents under environmental stresses has been reported. Our results are in agreement with those of EI-Tayeb, (2005) who found that carotenoid content decreased significantly in NaCl treated plants in comparison with untreated control. In plant tissues, the increased accumulation of amino acids under stress is related to protein function (Hussein *et al*., 2007). During current findings, it was found that leaf soluble proteins were increased by the application of aqueous extract at higher concentrations. Reduced level of leaf soluble proteins were found in wheat receiving 5% methanolic extract of both GM and non-GM maize plants. The leaf soluble protein decreased significantly with non GM maize extracts. Our results are in agreement with previous findings of Undovenko, (1971) who reported that the level of protein decreased with the stoppage of nitrate reeducates activity. Similarly, the level of soluble protein reduced in legume crops with treatment of *Acacia nilotica* extracts (Duhan & Lakshinarayana 1995). Our results are similar with those of Baziramakenga *et al.*, (1997) who reported that phenolic acids decreased the incorporation of certain amino acids into proteins and thus reduce the rate of protein synthesis. Maize has been reported to posse's three phenolic acids (Imran *et al*., 2006) which might have resulted in decreasing the protein content of wheat leaves. The phenolic acids have been shown to be toxic to the activities of many enzymes (Einhelling 1995).Our results are in agreement with those of EI-Darier& S.M, (1999) that maize plants accumulate different amino acids in response to the application of eucalyptus extracts. Proline is one of the most important water soluble amino acids and is supposed to play an important role in osmotic adjustment with regard to reduction of osmotic potential due to net accumulation of solutes (Handa *et al*., 1986; Raggi, 1994). Plants accumulate osmolytes like proline in response to Abiotec stresses to protect the macromolecules of cells (Hong *et al*., 2000 and Chutipaijit *et al*., 2009). Proline is also known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kishore *et al.,* 2005). Over accumulation of osmolytes may help plants to tolerate against stress by improving their ability to maintain osmotic balance within the cell (Apse & Blumwald 2002). The high level of proline may be protecting plants from stress conditions (Kumar *et al.,* 2003). Present investigation showed that accumulation of proline decreased significantly in wheat leaves in response to the application of both methanolic and aqueous extracts of GM maize leaves. Maximum non significant increase in proline was recorded with GM maize aqueous extracts at lower concentration (3%). The increase in proline content in response to allelochemicals has been reported. Abdulghadar & Nabat (2008) reported that with application of heliotrope leaves extracts, level of proline significantly increased in leaves of Dodder. The increased accumulation of proline content in response to drought and salt stresses has also been reported by Erdei *et al*., (2002) and Shao *et al*., (2006). The current investigation showed that increased accumulation of proline in response to aqueous extracts might revealed that aqueous extracts of both GM and non-GM maize plants gave some sort of osmotic stress to wheat which resulted in increased accumulation of stress oriented proline. The leaf soluble proline significantly decreased with aqueous extracts but methanolic extracts increased non significantly as compared with untreated control. The aqueous extracts of GM maize were highly inhibitory to leaf soluble sugars in wheat and the effect was more pronounced at higher concentrations of the extract. Previous studies conducted by El-Khawas & Shehata (2005) also established that phenolics present in the leaf extracts of *Acacia nilotica* inhibited the leaf sugar content of maize leaves in pot experiments. The inhibitory effects of aqueous extracts of GM maize on leaf soluble sugars of wheat might be due to the presence of greater concentrations of water soluble phenolics in the extracts. Many plants are reported to increase the level of antioxidant enzymes in response to environmental stresses because both biotic and Abiotic stresses are responsible for the production of reactive oxygen species (ROS) (Dat *et al*., 2000). The most common and earlier response of plants to drought, temperature, salinity, freezing and wounding etc is the accumulation of antioxidant enzymes like peroxidases (POD), superoxide dismutase (SOD) and Catalase (CAT) (Jiang & Zhang 2004). During current investigation, the level of antioxidant enzymes either increased or decreased in the leaves of wheat by the application of both methanolic and aqueous extracts. The application of methanolic and aqueous extracts of GM maize non significantly increased the level of super oxide dismutase (SOD) activity. Maximum significant increase in level of SOD was observed in response to the application of lower aqueous extract (3%) of GM maize and 5% methanolic extracts of non-GM maize as compared with untreated control. This shows that higher concentration of methanolic extracts exhibited greater effect on wheat plants as compared with aqueous ones. The increase in antioxidant activity in response to different stresses has been reported previously by Bor *et al*., (2003). Incensement in SOD activity in our results is same as reported by Gomez *et al*., (2004) who found an increase in all SOD enzymes of pea chloroplast following a long term of Nacl treatments. According to Koca *et al*., (2007) salinity leads to decrease in SOD activity in salt sensitive plants of *Sesame indicum* L. than salt tolerant ones (Akbar *et al*., 2012). The induction of peroxidase (POD) activity in plants occurs in response to many biotic and Abiotec stresses (Casal *et al*., 1994). Peroxidase (POD) is believed

to play an important roles in auxin catabolism, the oxidation of phenolics to form lignin, the cross linking of hydroxyl proline-rich glycol proteins in plant cell walls, and the production and breakdown of hydrogen peroxide and other reactive oxygen species (Klotz & Lagrimini 1996). The roles that POD can play in cell wall toughening and in the production of toxic secondary metabolites and its simultaneous oxidant and anti-oxidant capabilities can make it an important factor in the integrated defense response of plants to variety of stresses (Felton *et al*., 1989). The result of the present work indicated that POD activity decreased in wheat receiving both methanolic and aqueous extracts of GM maize. Maximum significant decrease in activity of POD was found with lower aqueous extract (3%) of GM maize. However, non-GM maize extract non-significantly increased the accumulation of peroxidase. Maximum non significant increase in peroxidase activity was recorded in succeeding crop receiving non-GM methanolic (5%) extract in comparison with untreated control. The GM methanolic extracts non significantly decreased the activity of Catalase in wheat but aqueous extracts enhanced the activity of Catalase in wheat crop. Methanolic and aqueous extracts of non-GM maize significantly increased the activity of Catalase. Maximum significant increase in Catalase activity was recorded with lower aqueous extract (3%) of non-GM maize as compared with untreated control. The increase in Catalase activity in response to biotic and Abiotec stresses has been reported. Previous study showed that Catalase and guaiacol peoxidase increased in two varieties of oat (Aramir & R567) under water stress .An increase in Catalase activity has been reported in other studies on allelochemicals mode of action, that is, furulic acid increased Catalase activity in maize seedlings (Dev & Prasad 1996) and benzoic acid in cucumber cotyledons (Maffei *et al*., 1999). From our results it can be inferred that non GM maize posses some allelochemicals which might have significantly increased the Catalase (CAT) activity in wheat crop (Fig. 1a-b; Fig. 2c-d; Fig. 3e-f).

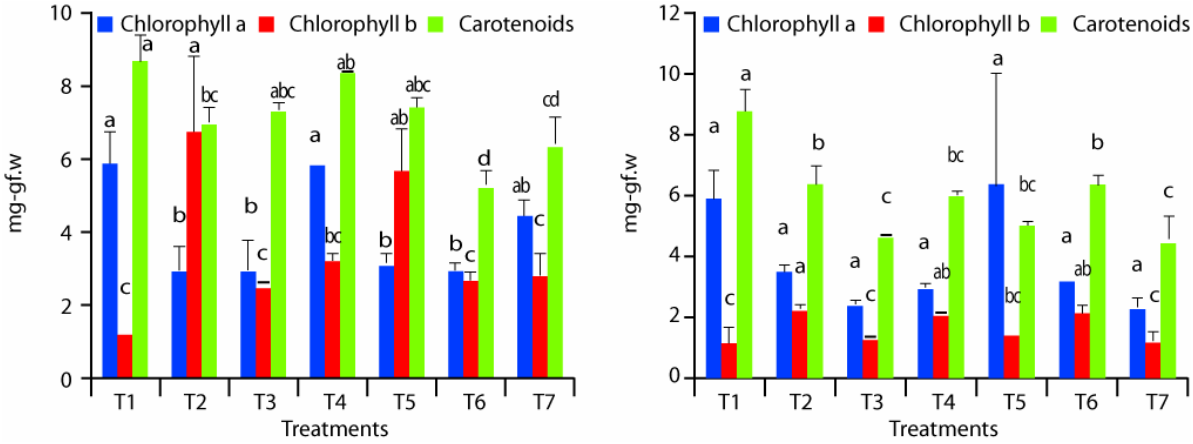

Fig. 1a-b. Effect of GM and non GM maize leaves methanolic and aqueous extract on chlorophyll and carotenoid content (mg ¯g f.w) of wheat cv.chakwall-97.

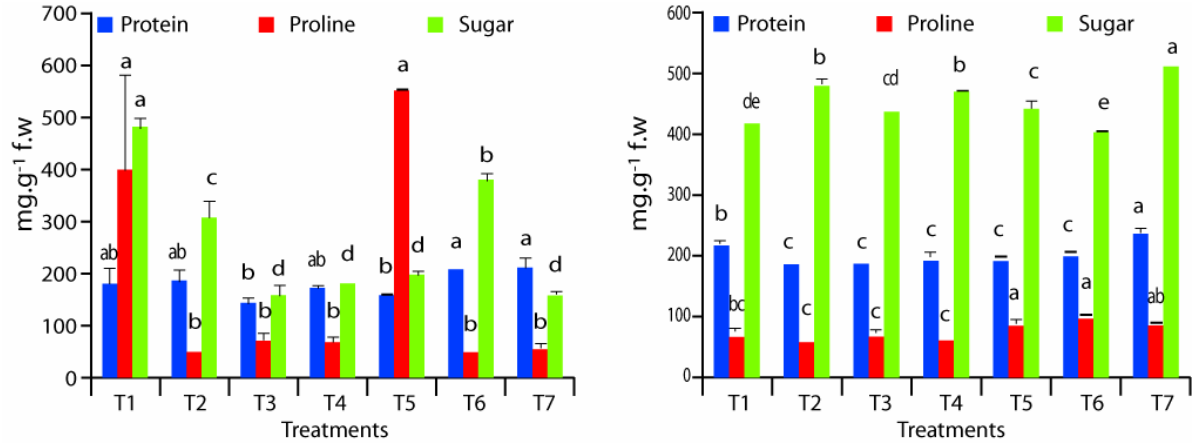

Fig. 2c-d. Effect of GM and non GM maize leaves methanolic and aqueous extract on Protein, Proline and Sugar content (mg ¯g f.w) of wheat cv.chakwall-97. Columns with same letters at the top are not significantly different (P< 0.05); error bars represent standard error. T1, Control; T2, Methanolic extract 3%; T3, Methanolic extract 5%; T4, Methanolic extract 10%; T5, Aqueous extract 3%; T6, Aqueous extract 5%; T7, Aqueous extract 10%

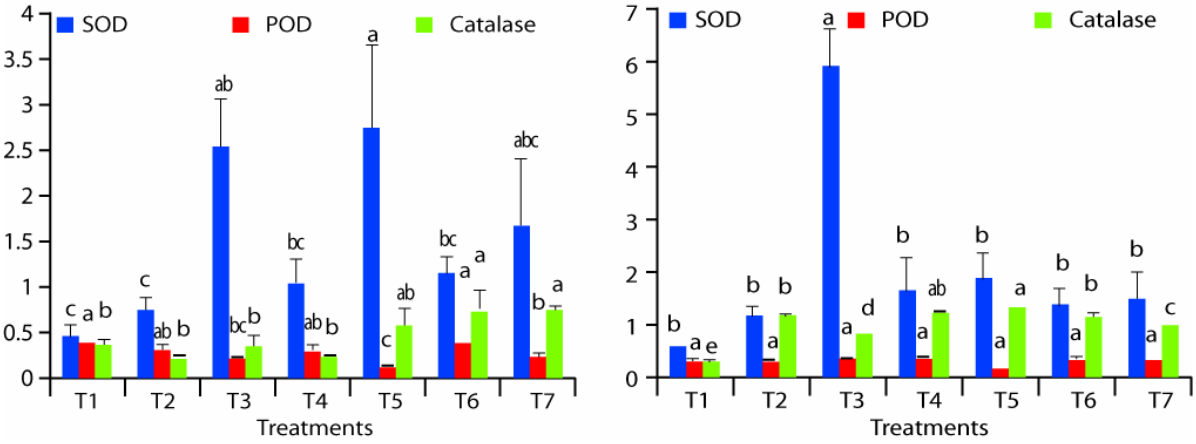

Fig. 3e-f. Effect of GM and non GM maize leaves methanolic and aqueous extract on Superoxide dismutase (SOD) (units/g f.w), Peroxidase (POD) and Catalase (CAT) content (units /min) of wheat cv.chakwall-97.Columns with same letters at the top are not significantly different (P< 0.05); error bars represent standard error. T1, Control; T2, Methanolic extract 3%; T3, Methanolic extract 5%; T4, Methanolic extract 10%; T5, Aqueous extract 3%; T6, Aqueous extract 5%; T7, Aqueous extract 10%

Conclusion

GM maize extracts increased the leaf contents of chlorophyll, soluble proteins and activity of SOD and catalase but decreased the contents of chlorophyll a, carotenoid, leaf soluble sugar, proline and activity of POD in wheat crop. While non-GM maize extracts significantly increased leaf content of chlorophyll b, soluble sugar and activity of catalase but decreased the content of chlorophyll a, carotenoid and leaf soluble proteins in succeeding crop wheat. The activities of SOD, POD and catalase were increased non-significantly in wheat crop as compared with untreated control.

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