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Full Length Article

Computation of Differential Response of Sunflower Genotypes for Achene Yield and Oil Quality against Lead Toxicity

Rizwana Qamar[1], Amir Bibi[1*], Hafeez Ahmad Sadaqat[1], Faisal Saeed Awan[2] and Hafiz Muhammad Akram[3]
[1]*Department of Plant breeding and Genetics, University of Agriculture Faisalabad, Pakistan*
[2]*Center of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad, Pakistan*
[3]*Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan*
[*]For correspondence: ameerbibi@gmail.com

Abstract

With increasing industrialization, soil contaminations with heavy metals especially lead (Pb) and its accumulation in edible crops is becoming a major concern. This research work was designed to find out differential response of sunflower genotypes against Pb toxicity and selection of Pb tolerant genotypes for utilization in future breeding program. Research trial was conducted in net house of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad. Fifty sunflower genotypes were collected from international sources and evaluated for 100-achene weight, achene yield per plant, oil contents, fatty acid profile and Pb contents in roots, shoots leaves and achene under three levels of Pb *viz*., 0, 150 and 300 mg Pb kg^{-1} of soil. Sunflower genotypes, Pb levels and interaction among them had significant effect on most of the aforementioned traits of sunflower. Principal component analysis was used for selecting better sunflower genotypes for achene yield and oil quality under Pb stress environments. Results disclosed that the genotypes PI546356, CN42267 and CN36537 were Pb tolerant while PI650582, CN36721 and CN31766 were Pb sensitive genotypes. Increasing lead concentration in various parts; roots, leaves and shoots had enormous negative influence on plant growth in general and on achene yield per plant in particular. Oleic acid (omega-9) contents increases with the decrease in linoleic acid (omega-6) contents with increasing Pb stress. Moreover, sunflower genotypes accumulated more amount of Pb in roots and leaves as compare to achenes, a required part for oil extraction. In conclusion, divergent response of sunflower genotypes under Pb stress and more importantly different degree of its accumulation in vegetative and reproductive parts, less accumulation in achenes in particular, provided the sound basis for establishment of Pb tolerant genotypes of sunflower.

Keywords: Genetic variability; Lead tolerance; Principal component analysis; Correlation; Oil contents; Fatty acid profile

Introduction

Environmental conditions which lower plant growth and yield below its optimum level are regarded as stress. Metallic elements with high density and atomic mass are termed as heavy metals. These are non-biodegradable elements which remains persistent in the environment (Jan *et al*., 2015). Soil pollution due to heavy metals has become an international issue as it has affected about 235 million ha of agricultural land globally (Bermudez *et al*., 2012). Farmers utilize waste from industries and cities to enhance organic matter and nutrients of their soil but this waste also contains several harmful metals (Mushtaq and Khan, 2010). These include lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As), nickel (Ni) and mercury (Hg) etc. Losses due to Pb toxicity can be assessed easily but there is no proper system of soil analysis in many areas of world and losses due to Pb stress remains ignored. Hence, pollution due to heavy metals, particularly Pb, becomes a risk factor not only for agriculture but also for the health of other living beings (Jalaluddin and Hamid, 2011; Khan *et al*., 2011). Stress posed by Pb causes numerous effects on plant physiological processes either directly or indirectly. Pb toxicity affects cell functioning, enzymatic reactions, nutrients uptake leading to chlorosis and declined photosynthesis ending with yield penalty (Sharma and Dubey, 2005; Shanker *et al*., 2005).

Addition of Pb in food cycle becomes hazardous for both plants and animals (Ahmad *et al*., 2013; Iqbal *et al*., 2017). Pb has small effects on metabolic processes, can cause brain disorders, coma, renal failure, cause death when exposed to high concentration (Papanikolaou *et al*., 2005) and interferes with proper development in children (Parashar and Prasad, 2013). Extended exposure to Pb may affect development and proper functioning of red blood cells, causing anemia. Metal contaminated food may exhaust reserves of essential nutrients in body thus weakening the defense mechanism of body against diseases, slow down growth and developmental processes and may cause disabilities related with malnutrition (Iyengar and Nair, 2000).

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Scientists had developed various economical techniques including employment of living plants and microorganisms for remediation of contaminated sites (Qiu *et al*., 2006). Plants have genetic potential to cope with high concentration of pollutants without showing toxicity symptoms. Researchers observed that some plant species can efficiently tolerate higher concentration of heavy metals or other toxic elements (Peralta *et al*., 2001). Indian mustard (*Brassica juncea* L.), corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were employed in phytoremediation studies as they showed high tolerance to heavy metals (Pilon-Smits, 2005).

Sunflower is a second major oilseed crop of Pakistan and has the ability to bridge up the gap between local production and oil import as government has to import oil from other countries worth Rs. 284.5 billion (Economic Survey of Pakistan, 2016–2017). Its oil has a premium quality with maximum concentration of unsaturated fatty acids (omega-6 & omega-9) along with good quantity of vitamins and antioxidants. Being a fast growing and high biomass crop, sunflower plant has the ability to accumulate toxic metals from soil. Details about status and arrays of genetic diversity are essential for wide applications in plant breeding. This type of studies highlights resemblances and variations in genetic resources (Reif *et al*., 2005) leading to organize grouping of gene banks and separation of remarkable parental combinations. The present study was therefore planned to evaluate genetic variability, genetic associations among economic traits and isolation of best performing genotypes based on achene yield and oil quality under Pb stress.

Materials and Methods

A research trial was organized in net house of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad during spring, 2015.

Experimental Details

Fifty genotypes of sunflower collected from United States National Germplasm System (US-NPGS) and Plant Gene Resources of Canada (PGRC; Table 1) were used as experimental material. All these genotypes were grown under three levels of Pb *i.e*., 0,150 and 300 mg Pb kg^{-1} of soil in soil filled pots using $Pb(NO_3)_2$ as source. The experiment was laid out following completely randomized design with three replications. The pots (with 25 cm height and 20 cm width) were filled with 12 kg Pb free, air dried, ground and sieved soil. The soil in pots was thoroughly mixed 6 times in a month to develop toxicity symptoms. Soil used in the experiment had 7.8 pH, 2.25 dS m^{-1} EC and 0.56% organic matter.

Crop Husbandry

Five seeds of all genotypes were sown under three levels of toxicity in separate pots. Only two healthy seedlings were kept and maintained till maturity after removing extra seedlings at 5–6 leaf stage. Standard agronomic and plant protection practices were followed during the conduct of whole trial. Recommended dose of NPK (118:85:62 kg/ha) were applied to pots. Pots were irrigated after 2 to 3 days according to weather conditions. Plants were uprooted after completion of physiological maturity (100–110 days).

Data Collection

Roots, shoots, leaves and achene were separated from plants through sharp cutting knife. Leaves and shoot samples were collected from three locations (bottom, middle, top) from a single plant and samples were prepared for digestion after oven drying at 80°C for 2 days. Plant samples were digested according to Ryan *et al*. (2001) with some modifications. After drying, samples were grounded to fine powder and 0.5 g of each sample was used for digestion using tri-acid method. Digestion was done on hot plate, keeping temperature 100°C for 1st h, 150ºC for 2nd h, 200°C for 3rd h and 250ºC for 4th h using 15 mL of digestion mixture. After digestion samples were filtered with Whatmann's filter paper No. 42 and volume was made accordingly by adding distilled water. The filtered samples were stored in air tight plastic bottles and subjected to heavy metal analysis in atomic absorption spectrophotometer (Model Thermo Electron S-Series).

Achene yield and 100-achene weight were recorded by using digital balance. One gram sample of achene from each genotype under all treatments were prepared and subject to Nuclear Magnetic Resonance (NMR) for determination of oil contents, oleic acid, linoleic acid and palmitic acid.

Statistical Analysis

Recorded data were subjected to analysis of variance (Steel *et al*., 1997) to estimate the presence of useful genetic variability through statistics 8.1 and genotypes were selected through GGE biplot analysis (Yan *et al*., 2000) by using computer software Microsoft Excel along with XLSTAT Version 2012.1.02, Copyright Addinsoft 1995-2012 (http://www.xlstat.com).

Results

Analysis of variance portrayed that sunflower genotypes had highly variable behavior for studied traits under different treatments of Pb toxicity. Highly significant effects of Pb treatments were also observed for all studied traits. Genotypes × Treatment interaction was also significant for all traits except 100-achene weight and oil contents (Table 2). Means of studied traits under control and two levels of Pb toxicity are presented in Fig. 1. Mean performance of sunflower genotypes for 100-achene weight, achene yield

Table 1: List of 50 sunflower genotypes collected from US-NPGS and PGRC

US-NPGS								PGRC	
No	Genotype	No	Genotype	No	Genotype	No	Genotype	No	Genotype
1	PI 497244	11	PI543743	21	PI650569	31	CN31766	41	CN36683
2	PI505837	12	PI543744	22	PI650572	32	CN33284	42	CN36685
3	PI509051	13	PI546356	23	PI650574	33	CN33285	43	CN36717
4	PI509052	14	PI566826	24	PI650582	34	CN33291	44	CN36721
5	PI536623	15	PI597371	25	PI650585	35	CN35799	45	CN42231
6	PI536624	16	PI599979	26	PI650592	36	CN36537	46	CN42267
7	PI536625	17	PI600724	27	PI650593	37	CN36582	47	CN42273
8	PI536626	18	PI600725	28	PI650594	38	CN36626	48	CN45042
9	PI536627	19	PI607508	29	PI650604	39	CN36629	49	CN45049
10	PI536629	20	PI617099	30	PI650608	40	CN36673	50	CN45074

Table 2: Mean squares of various traits of sunflower genotypes against Pb toxicity

Variables	Genotypes	Treatments	Interaction
Degrees of Freedom	49	2	98
100-achene weight (g)	4.68**	29.71**	0.019^{ns}
Achene yield per plant (g)	363.64**	3834.75**	4.05**
Oil contents (%)	17.11**	695.95**	9.3^{ns}
Oleic acid (%)	60.57**	306.73**	1.29**
Linoleic acid (%)	41.68**	306.73**	2.28**
Palmitic acid (%)	4.29**	7.93**	2.95**
Pb contents in roots (mg kg^{-1})	100.7**	99392**	26.7**
Pb contents in shoots (mg kg^{-1})	0.03**	12.14**	0.01**
Pb contents in leaves (mg kg^{-1})	44.4**	53364.7**	14.0**
Pb contents in achene (mg kg^{-1})	0.51**	148.33**	0.22**

** = Highly significant; ns = Non-significant

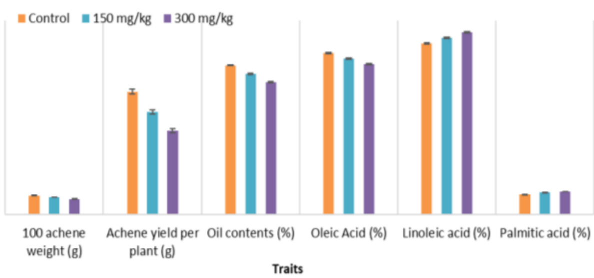

Fig. 1: Mean response of sunflower genotype for yield and oil quality related traits under lead toxicity

per plant, oil contents, oleic acid reduced whereas linoleic acid and palmitic acid increased with increasing toxicity levels. Mean accumulation of Pb in plant parts with increasing levels of Pb toxicity are shown in Fig. 2. Maximum accumulation of Pb was observed in roots followed by leaves of sunflower genotypes whereas very little concentration of Pb was accumulated in stem and achene. Sunflower genotypes accumulated higher concentration of Pb in its parts with the increase in concentration of Pb in soil.

Bi-plot analysis of sunflower genotypes along with character association under Pb stress is presented in Fig. 3. The principal components having more than 1 Eigen value

Table 3: Principle component analysis and main component values of analyzed sunflower genotypes under lead stress

	PC1	PC2	PC3
Eigen value	2.51	1.92	1.35
Proportion	0.30	0.26	0.11
Cumulative	0.25	0.44	0.58
Variables			
100Aw	-0.44	-0.19	-0.16
AY/P	-0.58	-0.15	-0.09
OC	0.03	0.16	0.021
OA	0.16	-0.62	0.064
LA	-0.19	0.56	0.03
PA	0.19	0.05	-0.28
PbR	0.09	-0.26	-0.56
PbL	0.30	-0.03	-0.43
PbS	0.06	-0.32	0.59
PbA	0.49	0.15	0.13

PC = Principal components, 100 Aw = 100-achene weight, AY/P = Achene yield per plant, OC = Oil contents, OA = Oleic acid, LA = Linoleic acid, PA = Palmitic acid, PbR = Lead contents in roots, PbL = Lead contents in leaves, PbS = Lead contents in shoots, PbA = Lead contents in achene

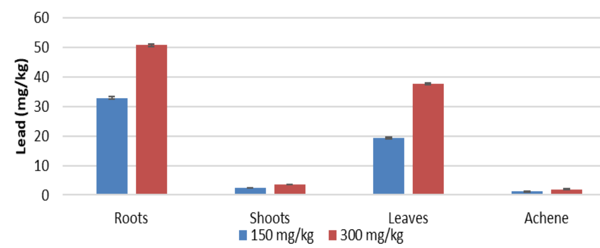

Fig. 2: Average accumulation of lead in 50 sunflower genotypes under lead toxicity

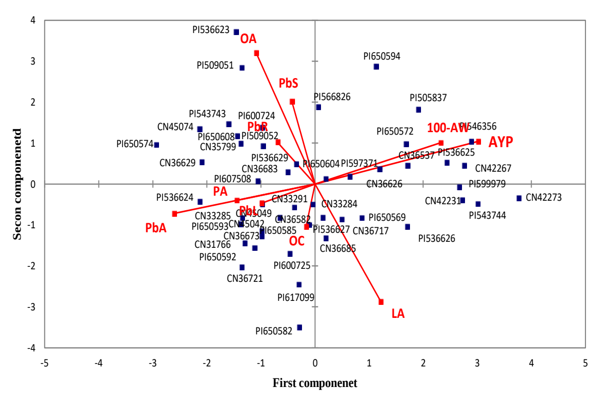

Fig. 3: Biplot analysis of 50 sunflower genotypes under Pb stress

under Pb stress are presented in Table 3. First three PCs contribute 25, 42 and 58% in total variability. In graphical presentation of biplot, genotypes that are present in positive quadrate are regarded as tolerant and genotypes in negative quadrate as susceptible ones. In this studies, Genotype PI546356, CN42267 and CN36537 were selected as Pb tolerant genotypes as they fall in positive quadrate whereas the genotypes PI650582, CN36721 and CN31766 falls in negative quadrate and regarded as Pb sensitive genotypes. Associations among various characters are very much important to develop selection criterion for the production of improved cultivars. Achene yield per plant had significant and positive association with 100-achene weight and negative association with palmitic acid under Pb stress. Oleic acid had negative association with linoleic acid. Highly significant and negative association of achene yield with Pb contents in roots, leaves and achene were observed. Accumulation of Pb in roots, leaves and achene had significant and positive association with palmitic acid and linoleic acid under Pb stress.

Discussion

Since the systematic plant breeding started, genetic variability within various traits have been identified in field crops and used for its improvement. In existing picture of climate change and pollution, computation of genetic variability provides opportunity to plant breeders for utilization of yield contributing characters and their association in development of better cultivars with improved yield and quality features under stress environments. Keeping seed yield and oil quality in view, statistical analysis showed that all traits varied significantly among studied genotypes. Stress treatments of Pb also had highly noticeable effects on studied traits. Moreover, all characters were highly affected by interaction of genotypes and treatments except for 100-AW and oil contents. Neto *et al*. (2016) also reported significant G × E interaction for fatty acid composition of sunflower oil.

Exposure to Pb stress reduced achene yield, 100-achene weight and oil contents in sunflower genotypes. Lavado (2006) also reported significant reduction in achene yield per plant in sunflower. Disturbances in plant cellular functioning had negative impact on economically important traits. Excess of Pb had phytotoxic effects on growth and developmental processes in sunflower (Paliwal *et al*., 2014; Awan *et al*., 2015). Moreover, heavy metals are also linked with increased production of reactive oxygen species and other cytotoxic compounds which disturbs cellular homeostasis (Sytar *et al*., 2013). Fatty acid profile of oil determines its use (Metzger and Bornscheuer, 2006). Traditional sunflower genotypes had high concentration of unsaturated fatty acids (oleic acid & linoleic acid) and low concentration of saturated fatty acids (Palmitic acid) in its oil (Neto *et al*., 2016). Oil having high linoleic acid is good for edible purposes whereas high oleic acid oil has its uses in industry (Haddadi *et al*., 2011). Lead stress had strong impact on fatty acid profile in sunflower oil by altering its fatty acid ratio. The concentration of oleic acid decreased whereas the concentration of linoleic acid and palmitic acid increased.

Heavy metals accumulation in plant organs depends upon both internal (plant associated) and external (soil associated) factors. Plants can uptake heavy metals through proton pumps, ionic channels and transporter proteins and evapotranspiration presents the main force for translocation of metals to the above ground parts (Tangahu *et al*., 2011). Maximum accumulation of Pb was observed in plant roots than other parts (root>leaves>stem>seed) (Jadia *et al*., 2008; Paliwal *et al*., 2014) as roots are main tissues for storage of metal ions (Arsenijevic-Maksimovic *et al*., 2001) from contaminated environment which was then translocated to other tissues. Limited amount was transferred to shoots (Lagriffoul *et al*., 1998) because upon entrance in plasma membrane of root cells, metals precipitate, inactivates and form slowly movable compounds with organic matter. Moreover, concentrations of metals are reported to be more in epidermis than root cortex as the endodermis presents barrier to ion transport (Hagemeyer and Breckle, 1996). Trace amounts of Pb were observed in achene of sunflower (Sewalem *et al*., 2014; Angelova *et al*., 2016) and literature had supported the fact that metallic ions were not translocated to oil of sunflower after seed processing (Angelova *et al*., 2011, 2016) which makes it suitable for cultivation at metal contaminated soils with little health risk.

GGE biplot analysis assists in selection of desirable genotypes from a large set of genotypes on the basis of numerous traits. Isolation of Pb tolerant genotypes provided a concrete background for their further utilization in development of new genetic combinations or they can be directly used for cultivation on Pb contaminated soils. Identification of Pb sensitive genotypes may be employed in studying genetic basis of Pb tolerance in sunflower. It is therefore suggested that genotypes with wider genetic variation complemented with desirable features could be efficiently engaged in intra specific crosses with the hope that this would lead to the transmission of higher genetic gain for desirable traits especially yield. Biplot has been used by many researchers to select genotypes on the basis of their performance in different testing environments (Balalic *et al*., 2012; Brankovic *et al*., 2012).

Similarly, knowledge about significant relationship among characters is important for launching an effective breeding program as it offers a chance for selection of appropriate genotypes with desirable traits simultaneously (Ali *et al*., 2009). Correlation studies had revealed some important associations among yield and oil quality related parameters under Pb toxicity. Achene yield had significant and positive relationship with 100-achene weight and negative association with Pb contents in roots, leaves and achene. Negative association between oleic acid and linoleic acid (Onemli, 2012; Neto *et al*., 2016) suggested that these two important fatty acids cannot be improved simultaneously. Heavy metals stress reduced the oil contents and altered the fatty acid composition of sunflower oil (Angelova *et al*., 2016). Moreover, oleic acid content decreased while palmitic acid content increased upon the exposure to heavy metals (Khan *et al*., 2013). Aguirrezabal *et al*. (2015) develop conceptual models, which suggested that change in oil and fatty acid contents may be due to the function of carbohydrates. Stress conditions lower distribution of assimilates to grains which effects biosynthetic pathway of oil and fatty acids. This relationship clearly suggested the importance of these parameters for selection of Pb tolerant genotypes for their future utilization

Conclusion

Increasing Pd stress impaired the achene yield and oil quality of sunflower; however different genotypes behaved differently under stressful environment. The genotypes PI546356, CN42267 and CN36537 were fund Pb tolerant while PI650582, CN36721 and CN31766 were Pb regarded as sensitive ones. Oleic acid (omega-9) contents increased on the expense of linoleic acid (omega-6) contents with increasing Pb. Moreover, sunflower genotypes accumulated more amount of Pb in roots and leaves as compare to achenes, a required part for oil extraction.

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