Zagazig J. Agric. Res., Vol. 44 No. (2) 2017 635-642

Agricultural Biochemistry and its Application

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

CHARACTERIZATION OF TOLERATE BIOMARKER IN FABA BEAN (*Vicia faba* L.) AGAINST *Orobanche crenata*

Alaa E. Abosaif[1*], A. Sharaf[1], R.A. Elmassry[2] and M.A. Doheim[2]

1. Agric. Biochem. Branch., Soil and Water Sci. Dept., Fac. Technol. and Deve., Zagazig Univ., Egypt

2. Agric. Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 05/02/2017 ; Accepted: 05/03/2017

ABSTRACT: This study was conducted in Zagazig University, Egypt to evaluate tolerate biomarkers in faba bean against *Orobanche crenata*. Two faba bean genotypes were choicen, known as Msr-3 (tolerate) and Giza-716 (susceptible). Obtained results showed that tolerate genotype has high content of chlorophyll a (1.20 mg/ g fresh weight), chlorophyll b (0.69 mg/g fresh weight), total chlorophyll (1.89 mg/ g fresh weight) and carotenoid (0.97 mg/ g fresh weight). Also tolerate faba bean contained high content of componants known as major osmotically-active componants, namely total soluble sugar (0.045 mg/ml), total protein (7.85%), Na^+ (39.16ppm), K^+ (59.24ppm) and polar amino acids (78.54%). Tolerate faba bean also showed a high content of total phenolic compounds (348.84 ppm calculated as a Gallic acid), conjugated phenolic compounds (227.69 ppm calculated as a Gallic acid). As well as tolerate faba bean (Msr-3) has high catalase activity (0.39 mg. $protein^{-1}$).

Key words: Faba bean, broomrape, soluble sugars, protein, phenolic compounds, cations and catalase.

INTRODUCTON

Vicia faba L. (Faba bean) is a plant related to Leguminosae family. These species play important biological role which is considered a good source of carbohydrate fraction, protein fraction, antioxidant, lipids and minerals for both humans and animals. The chemical analysis of seeds by Haciseferogullari *et al*. (2003) and Khalil *et al*. (2015) showed that seeds contain 35% protein, 45% carbohydrate and 2% fat. Lindemann and Glover (2003) stated that faba bean cultivation improves soil fertility, where it is fixing atmospheric nitrogen through biotical N_2-fixation.

FAO (2013) illustrated that, in Egypt, cultivated area through seasons 2006 and 2011 were 147.000 faddan with mean yield 8.8 ardabs/ faddan.

Orobanche crenata is considered most faba bean root parasite in Egypt, sense; it is causing great losses in yield but sometimes complete losses of the crop in endemic land. Also, one of the most important factors reducing faba bean yield is the infestation of the crop with broomrape as reported by Abo El-Kheir *et al*. (2010).

Tank *et al*. (2006) and Joel (2009) in their studies considered *Orobanche crenata* (broomrapes) is one of most obligate plant-parasitic plants from the genera Orobanche and Phelipanche in the Orobanchaceae family. Control, evaluation and the resistance of the parasitic weed are most difficult, since tolerance against broomrapes was identified so far as polygenic nature according by Diaz-Ruiz *et al*. (2009).

One of the most important goals of breeding programs is developing genotype that resists broomrape weed. In spite of that Gillanders *et al*. (2002) stated that identification of tolerate biomarkers in resistant faba bean cultivars against broomrapes can help these breeding

* **Corresponding author: Tel. :** +201003702174
E-mail address: alaaabosaif@Zu.edu.eg

programs to tagging the important traits. So Abbes *et al*. (2009) found that the deficiency of nitrogen content in phloem exudates of faba bean genotype correlated with a tolerance against *Orobanche crenata*. Also they stated that potassium and calcium cations as osmotically-active agents in both tubercles and shoots correlated with tolerate faba bean. The same authors noticed shoots and tubercles of faba bean accumulated hexoses, starch, aspartic acid and asparagine help faba bean plants to tolerate broomrape weed. Beckman (2000) investigated phenolic compounds content in tolerate faba bean and stated that the synthase and strategic location of phenolic compounds are accompanying with tolerance against *Orobanche crenata*. Also Verkleij *et al*. (1991) and Gadalla *et al.* (2012) reported that tolerate faba bean characterization by high activities of peroxidase and polyphenol-oxidase isozymes than susceptible faba bean.

So this study was conducted to evaluate and compare of some tolerate biomarkers of tolerate and susceptible faba bean.

MATERIALS AND METHODS

Materials

The present investigation was carried out during the two successive seasons of 2013 and 2014 in green house in Faculty of Technology and Development, Zagazig University, Egypt. Two cultivars (Msr-3) which known tolerate genotype and (Giza-716) which known susceptible genotype against *O. crenata* were obtained kindly from Agricultural Research Centre, Giza, Egypt. Pots (50 cm in dimeter) filled with a clay soil were subjected for each cultivar. The pots were pre fertilizer with super phosphate, potassium sulfate and ammonium sulfate. Seeds of faba bean were sown at 3cm from the soil surface (6 seeds/pot). Samples were taken two times, 45 and 90 days after planting for the both cultivars, at every stage the plant samples were taken for chemical analysis and surrounding soil of the roots were taken for pH and EC analysis.

Methods

Soil pH

Soil pH was measured in the soil water suspension 1:2.5 according to the method of Jackson *et al*. (1973).

Electrical conductivity EC

Electrical conductivity EC was measured in the soil in 1: 5 soil extract according to the methods of Cottenie *et al*. (1982) and Page *et al*. (1982).

Agronomic traits

Agronomic traits which determined were number of leaves, fresh weight (g), shoot weight (g), root weight (g) and root/shoot ratio.

The photosynthetic pigments

The photosynthetic pigments were determined according to the method of Wettestein (1957).

Carbohydrate fractions

The concentration of total soluble sugars, reducing sugars and non-reducing sugars were determined according to Miller (1959).

Total protein

Total protein was determined according to method of AOAC (2002).

Sodium and potassium

Sodium and potassium concentrations were determined according to Allen *et al.* (1974).

Phenolic compounds

Phenolic compounds were determined using colorimetric methods as described by Snell and Snell (1954).

Catalase activity

Catalase activity was assayed according to the method of Kato and Shimizu (1987). The enzyme activity was calculated according to the following equation,

$$\text{Enzyme activity [unit (mg. protein)}^{-1}] = K \times (\Delta A/\text{min.})$$

Where:

K (extension coefficient) is 40 mM / cm at 240 nm for H_2O_2. ΔA/min is the change in absorbency per minute.

Polyphenol oxidase activity

Polyphenol oxidase activity was determined according to Esterbaner *et al*. (1977). The activity of polyphenol oxidase was expressed according to the following equation,

Enzyme activity [unit (mg. protein)$^{-1}$] =
K × (ΔA/min).

Where:

K (extension coefficient) is 0.272 mM/cm at 490 nm for catechol. ΔA/min is the change in the absorbance of the mixture every 0.5 minute for 5 minutes period at 490 nm.

Amino acids

The amino acids content was determined by using an Automatic Amino Acid Analyzer (Model: AAA 400 INGOS Ltd) according to the method of Csomos and Simon-Sarkadi, 2002).

RESULTS AND DISCUSSION

Results in Table 1 show that there is no drastically changes in both soil pH or EC values during growth stages of Msr-3 (tolerate) and Giza- 716 (sensitive) against *O. crenata*. While there are some differences in soil EC values. Since EC of soil after 45 days of sowing as well as after 90 days of sowing with Msr-3 (tolerate) was higher than that of Giza- 716 (0.66, 0.45 and 0.51, 0.42), respectively.

The results in Table 2 show that susceptible cultivar (Giza- 716) had higher morphological parameters than those of tolerate cultivar (Msr-3), these may be due to the high content of water, also photosynthesis process is depending not only on the number of leaves but also on the surfer area of leaf and photosynthetic pigments as it is showen in Table 2.

Results illustrated in Table 3 clarify that Msr-3 (tolerate) had a higher content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid (1.20, 0.69, 1.89 and 0.97 mg/g fresh wt. respectively) than those of Giza-716 (susceptible) (0.83, 0.61, 1.44 and 0.75 mg/g fresh wt. respectively) that decrease of nitrogen content in cytosol, since nitrogen deficiency is correlated with high tolerance as stated by Gillanders *et al*. (2002). In the response of carbohydrates, it can be noticed in Table 4 that tolerate faba bean (Msr-3) was contained higher contents of total soluble sugars, non-reducing sugar and total protein through growth stages (0.045, 0.0217 mg/ml 7.85 % after 45 of sowing, respectively) and (0.0524, 0.0265 mg/ml 6.41% after 90 of sowing, respectively) than those of susceptible faba bean (0.035, 0.0124 mg/ml 7.13 after 45 days of sowing, respectively) and (0.0508, 0.0233 mg/ml 7.13 after 90 days of sowing, respectively). Tolerance correlated with high content of organic solutes as stated by Abbes *et al*. (2009). Also the high content of total protein in tolerate faba bean may be due to the fast change of inorganic nitrogen (ammonia and nitrate) to protein that decrease inorganic nitrogen in the phloem of tolerate faba bean as stated by Abbes *et al*. (2009).

As shown in Table 5 the cations content, namely Na^+ and K^+ in tolerate faba bean (Msr-3) showed higher values of both Na^+ and K^+, especially at first stage of growth (*i.e.* after 45 days of sowing, since they amounted 39. 16 and 59.24 ppm, respectively) than susceptible faba bean (16.25 and 47.83 ppm, respectively). These predominated cations are the major osmotically active compounds in both tubercles and shoots as reported by Abbes *et al.* (2009). Phenolic compounds are synthesizing and storing in the vacuoles of plants, especially during differentiation processes that accompanied by high tolerance against *Orobanche crenata*. Results of Phenolic compounds determination in roots of both tolerate and susceptible faba bean were shown in Table 6. It can be noticed that Msr-3 (tolerate) contained higher concentration of total phenolic compounds (348.84 ppm calculated as a gallic acid), as well as conjugated phenolic compounds (277. 69 ppm calculated as a gallic acid), especially through first stage of growth, (process of differentiation) after 45 days of sowing than those of Giza- 716 (susceptible), 327.30 ppm and 327.30 ppm (calculated as a gallic acid). Also it can be noticed that Giza-716 (susceptible) contained high concentration of free phenolic compounds, 100 ppm and 197.31 ppm (calculated as a gallic acid) through all stage of growth (after 45 and 90 days of sowing, respectively).

Table 1.The mean differences of soil pH analysis and EC analysis (dS/m) of the variety (Msr-3) and variety (Giza- 716).

Variety	Stage- 2 (at 45th day)		Stage3 (at 90th day)	
	pH	EC	pH	EC
Msr-3	7.9	0.66	8.1	0.45
Giza-716	8.0	0.51	8.1	0.42

Table 2. Mean of morphological contents of variety (Msr-3) and variety (Giza- 716) at 90th days after planting

Variety	Mean of leaves number	Mean of fresh weight (g)	Mean of shoot weight(g)	Mean of root weight (g)	Root/ Shoot ratio
Msr-3	35.5	34.4	31.97	2.27	0.07
Giza-716	39.16	37.23	34.54	2.24	0.065

Table 3. Mean of photosynthetic pigment fractions of variety (Msr-3) and variety (Giza-716) at 90th days after planting (mg/g fresh Wt.).

Variety	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoid (mg/g)	Chlorophyll /carotenoid
Msr-3	1.20	0.69	1.89	0.97	1.94
Giza-716	0.83	0.61	1.44	0.75	1.92

Table 4. Mean of carbohydrate fractions (mg /ml as glucose) and total protein (%) of variety (Msr-3) and variety (Giza-716) at 45th and 90th days after planting

	Total soluble sugar (mg /ml as glucose)		Reducing sugar (mg /ml as glucose)		Non-reducing sugar (mg /ml as glucose)		Total protein (%)	
Variety	45thday	90thday	45th day	90thday	45th day	90thday	45thday	90thday
Msr-3	0.045	0.0524	0.0233	0.0259	0.0217	0.0265	7.85	6.41
Giza716	0.035	0.0508	0.0231	0.0275	0.0124	0.0233	7.13	5.78

Table 5. Mean of Na, K contents (ppm) and K/Na ratio in roots of Msr-3 (tolerate) and Giza-716 (susceptible) after 45 and 90 days of planting

Variety	Na (ppm)		K (ppm)		K/Na ratio	
	45thday	90thday	45thday	90thday	45th day	90thday
Msr-3	39.16	16.94	59.24	65.22	1.51	3.85
Giza716	16.25	15.55	47.83	83.15	2.94	5.34

Table 6. Mean of Phenolic compound (ppm calculated as a Gallic acid) contents in roots of Msr-3 (tolerate) and Giza-716 (susceptible) after 45 and 90 days of planting.

	Total phenolic compounds (ppm)		Free phenolic compounds (ppm)		Conjugated phenolic compounds (ppm)	
Variety	45th day	90thday	45th day	90thday	45th day	90thday
Msr-3	348.84	334.23	71.15	73.46	277.69	260.77
Giza716	327.30	403.46	100.00	197.31	227.30	206.15

These results (Table 6) indicated total phenolic compounds and conjugated phenolic compounds are more correlating with tolerance, as stated by Beckman (2000).

Since tolerance against *O. crenata* is correlating with enzyme activity as reported by Gadalla *et al*. (2012). Results in Table 7 show that catalase activity of tolerate faba bean roots (Msr-3) was higher (0.39 mg.protein^{-1}) than catalase activity of susceptible faba bean roots (Giza-716) (0.30 mg.proten^{-1}). Results in Table 7 also show that there were no differences between polyphenol oxidase activity in tolerate faba bean roots (Msr-3) and its activity in susceptible faba bean roots (Giza-716), (0.0013 mg.protein-1). This may be due to the time of evaluation of enzyme activity which occurred in the late stage of growth (after 90 days of planting).

The state of amino acids in both tolerate faba bean (Msr-3) and susceptible faba bean roots (Giza-716) was illustrated in Table 8. It can be noticed that Msr-3 contained higher percentage of most polar amino acids, such as, aspartic acid, serine, theronine, histadine and lysine (15.69, 2.81, 1.96, 1.31 and 56.77, respectively) (*i.e.* 78.54% of detected amino acids) than those of susceptible faba bean roots (Giza-716), since it contained 70.41% of detected amino acids. These amino acids were considered of major osmotically active compounds and good correlated with tolerance against broomrape, as reported by Abbes *et al*. (2009).

Also, tolerate faba bean (Msr-3) characterized by a high percentage of tryptophan (0.97%) that is considered a good precursor of auxins biosynthesis, as well as a high percentage of phenylalanine (1.77%) which known as a biochemical intermediate of antioxidant synthesis.

Table 7. Mean activity of enzymes (mg. protein^{-1}) in roots of Msr-3 (tolerate) and Giza-716 (susceptible) after 90 days of planting

Variety	Catalase activity	Polyphenol-oxidase activity
Msr-3	0.39	0.0013
Giza-716	0.30	0.0013

Table 8. Amino acids (%) in roots of Msr-3 (tolerate) and Giza-716 (susceptible) after 90 days of sowing

Amino acid	Relative quantitative (%)	
	Msr-3	Giza-716
Aspartic acid (Asp)	15.69	10.23
Theronine (Thr)	1.96	1.41
Serenine (Ser)	2.81	2.01
Glutamic (Glu)	0.00	0.00
Proline (Pro)	0.00	0.00
Glysine (Gly)	3.73	3.81
Alanine (Ala)	0.38	0.06
Valanine (Val)	7.98	8.87
Methionine (Met)	2.98	1.86
Isoleucine (Ile)	1.32	0.78
Leucine (Leu)	2.34	2.16
Tryptophane (Tyr)	0.97	0.55
Phenyllanine (Phe)	1.77	1.49
Histadine (His)	1.31	1.23
Lysine (lys)	56.77	65.53
Arginine (Arg)	0.00	0.00
Polar amino acids	78.54	70.41

REFERENCES

Abbes, Z., M. Kharrat, P. Delavault, W. Chaïbi and P. Simier (2009). Nitrogen and carbon relationships between the parasitic weed *Orobanche foetida* and susceptible and tolerant faba bean lines. Plant Physiol. and Biochem., 47 (2): 153-159.

Abo El-kheir, Z.A., M.S. Abdel-Hady, H.M.H. El-Naggar A.R. Abd El-Hamed (2010). Molecular and Biochemical Marker of Some *Vicia faba* L. Cultivars in Response to Broomrape Infestation. Nat. and Sci., 8 (11): 252-260.

Allen, S.E., H.M. Grimshaw, J.A. Parkinson and C.L. Quarmby (1974). Chemical analysis of ecological materials. Blackwell Scientific Publications.

AOAC (2002). Association of Official Analytical Chemists, Official Methods of Analysis, 17th Ed., Washington, DC, USA.

Beckman, C.H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants. Physiol. and Molecular Plant Pathol., 57 (3): 101-110.

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck (1982). Chemical Analysis of Plants and Soil", Lab. Agrochem, State Univ., Ghent, Belgium.

Csomos, E. and L. Simon-Sarkadi (2002). Characterization of tokaj wines based on free amino acid and biogenic amine using ion-exchange chromatography. Chromatographia Supplement, 56: 185−188.

Díaz-Ruiz, R., A.M. Torres, Z. Satovic, M.V. Gutierrez, J.I. Cubero and B. Román (2009). Validation of QTLs for *Orobanche crenata* resistance in faba bean (*Vicia faba* L.) across environments and generations. Theoretical and Appl. Genet., 120(5): 909-919.

Esterbaner, H., E. Schwarzl and M. Hayn (1977). In: Principles of Biochemistry (Publisher: Freeman. WH): 477- 486.

FAO (2013). Statistical year book, World food and agriculture. Food and Agric. Organization of the United Nations, Rome, 567.

Gadalla, N.O., E.M. Fahmy, A. Abd-Elsattar, N.A. Ashry, M.A. El-Enany and A. Bahieldin (2012). Evaluation of Gene Expression for *Orobanche* Tolerance in Faba bean (*Vicia faba* L.). J. King Abdulaziz Univ., 24 (1): 21.

Gillanders, E.M.A.B., B.S. Julia and W. Elizabeth (2002). Responses to Abiotic stress (Acadmic Press).

Haciseferogullari, H., I. Gezer, Y.C.H.O. Bahtiyarca and H.O. Mengeş (2003). Determination of some chemical and physical properties of Sakız faba bean (*Vicia faba* L. Var. major). J. Food Eng., 60 (4): 475-479.

Jackson, M.L., S.Y. Lee, J.L. Brown, I.B. Sachs and J.K. Syers (1973). Scanning electron microscopy of hydrous metal oxide crusts intercalated in naturally weathered micaceous vermiculite. Soil Sci. Soc. Ame. J., 37 (1): 127-131.

Joel, D.M. (2009). The new nomenclature of Orobanche and Phelipanche. Weed Res., 49 (s1): 6-7.

Kato, M. and S. Shimizu (1987). Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. Canadian J. Bot., 65 (4): 729-735.

Khalil, N.A., W.A. Al-Murshidy, A.M. Eman and R.A. Badawy (2015). Effect of plant density and calcium nutrition on growth and yield of some faba bean varieties under saline conditions. Int. Scient. Publications J., (3): 440-450.

Lindemann, W.C. and C.R. Glover (2003). Nitrogen fixation by legumes. Guide A-129, Coll. Agric. and Home Econ., New Mexico State Univ., 1-4.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chem., 31(3): 426-428.

Page, A.L., R.H. Miller and D.R. Keeny (1982). Methods of soil analysis, Part 2, Chemical and Biological properties, Ame. Soc. Agron., Inc.Publisher, Madison, Wisconsin, USA.

Snell, F.D. and C.T. Snell (1954). Colorimetric Methods of Analysis, 4.

Tank, D.C., P.M. Beardsley, S.A. Kelchner and R.G. Olmstead (2006). Las Johnson Review No. 7. Review of the systematics of Scrophulariaceae 1st and their current disposition. Aust. Systematic Bot., 19 (4): 289-307

Verkleij, J.A.C., W.S. Egbers and A.H. Pieterse (1991). Allozyme variations in populations of *Orobanche crenata* from Syria. Progress in Orobanche Res., 304-317.

Wettstein, D. (1957). Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. Exper. Cell Res., 12 (3): 427-506.

تحـــديـــد المعلمــــات الحيـــوية لقـــــوة التحمــــل فــــى الفــــول البلـــدى ضــــد الهــالــــوك

علاء عويس أبوسيف[1] ـ عبدالعزيز شرف[1] ـ رجب عبدالفتاح المصري[2] ـ محمود عبدالرازق دهيم[2]

١ـ قسم علوم الأراضى والمياة ـ فرع الكيمياء الحيوية ـ كلية التكنولوجيا والتنمية ـ جامعة الزقازيق – مصر

٢ـ قسم الكيمياء الحيويه الزراعية ـ كلية الزراعة ـ جامعة الزقازيق – مصر

أجريت هذة الدراسة فى جامعة الزقازيق (مصر) لتقييم المعلمات الحيوية فى الفول البلدى ضد الهالوك حيث تم إختيار الطراز الجينى مصر-٣ (مقاوم) والطراز الجينى جيزة- ٧١٦ (حساس)ـ أوضحت النتائج المتحصل عليها أن الطراز المقاوم (مصر-٣) ذو محتوى عالٍ من الصبغات النباتية (كلوروفيل-أ، كلوروفيل-ب والكلوروفيل الكلي وكذلك الكاروتينيدات ـ ١,٢٠ ـ ٠,٦٩ ـ ١,٦٩ ـ ٠,٩٧ ملليجرام/جرام على التوالى)، أيضا كان الطراز الجينى المقاوم (مصر-٣) ذو محتوى عالٍ من المركبات الأساسية فى النشاط الأسموزي مثال السكريات الذائية الكلية (٠,٠٤٥ مجم/مل) والبروتين الكلي (٧,٨٥%) وكاتيون الصوديوم (٣٩,١٦جزء في المليون) وكاتيون البوتاسيوم (٥٩,٢٤ جزء فى المليون) وأحماض أمينية قطبية (٧٨,٥٤ %) كما أوضحت النتائج أيضا أن مصر- ٣ كان ذو محتوى مرتفع من المركبات الفينولية الكلية (٣٤٨,٨٤ جزء فى المليون كحمض جاليك) ومركبات فينولية مرتبطة (٢٧٧,٦٤ جزء فى المليون كحمض جاليك) كما تميز مصر-٣ بنشاط مرتفع لإنزيم الكاتاليز (٠,٣٩ ملليجرام.بروتين$^{-1}$).

المحكمـــــون:

١ـ أ.د. فاروق جندى معوض	أستاذ الكيمياء المتفرغ ـ كلية الزراعة ـ جامعة عين شمس.
٢ـ أ.د. سيد سليمان السعدنى	أستاذ الكيمياء المتفرغ ـ كلية الزراعة ـ جامعة الزقازيق.