ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2015: 3 (2): 119–125

Invader Shoots with Invaded Roots on Diallel Analysis of Oriental Tobacco Genotypes under Egyptian Broomrape Stress

Reyhaneh Seyyed–Nazari[1] Mortaza Ghadimzadeh[1*] Reza Darvishzadeh[1] Seyyed Reza Alavi[2]

[1]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University. Urmia/Iran.
[2]Urmia Tobacco Research Centre (UTRC). Urmia/Iran.
[*]Corresponding author: m.ghadimzadeh@urmia.ac.ir

Geliş Tarihi: 08.02.2016 Kabul Tarihi: 29.02.2016

Abstract

Egyptian broomrape (EBR) (*Orobanche aegyptiaca* Pers. is one of the most dangerous parasitic plants for a broad spectrum of hosts including tobacco (*Nicotiana tabaccum* L.). Finding genotypes resistant to EBR in Oriental tobacco, one of the main types of tobacco is of great importance and is one of the breeding aims in fighting against this parasite in Iran. In this strategic direction, a research work using diallel analysis was conducted to identify resistant Oriental tobacco genotypes against EBR at Urmia Tobacco Research Centre, Iran in 2012. Fifteen F1 progenies along with their six parents were planted in randomized complete block design with three replications in two separate experiments. Analysis of variance of investigated traits [fresh weight of root (FWR) and dry weight of root (DWR) for host plants, and fresh weight of broomrape shoot (FWBS), dry weight of broomrape shoot (DWBS) and number of broomrape shoots (NBS) for parasite plants] using diallel analysis showed significant mean squares of specific combining ability (SCA) for DWR, DWBS and NBS indicating the importance of non-additive genetic effects for these traits. Good and poor general combiners were not determined among parents for investigated traits due to non-significance of general combining ability (GCA). A set of hybrids appeared to be significant SCA, which could be used in Oriental tobacco improvement.
Key Words: Diallel, Gene effects, GCA, SCA, Oriental tobacco, *Orobanche aegyptiaca* Pers.

Öz

Şark Tipi Tütün (*Nicotiana tabaccum* L.) Genotiplerinde Mısır Canavar Otu (*Orobanche aegyptiaca* Pers.) Bitkisince Saldırıya Uğramış Köklerin Kıyaslanması ve Diallel Analizleri

Mısırlı canavar otu (*Orobanche aegyptiaca* Pers.) bitkisi, parazit bitkiler içerisinde en tehlikeli türlerden olup, tütün (*Nicotiana tabaccum* L.) gibi birçok bitkiyi olumsuz yönde etkilemektedir. İran'da, şark tipi tütünlerde bu parazit bitkiye dayanıklı olanlarını bulmak, ıslah çalışmalarında en önemli misyonlardan birisidir. Bu araştırma, bu hedefe ulaşabilmek için Urumiye Tütün Araştırma Merkezi'nde kurulmuştur. Denemede, diallel analizleri kullanılarak, dayanıklı genotiplerin belirlenmesi hedeflenmiştir. Deneme, 15 tane melez ve 6 tane ebeveyni kıyaslamak amacıyla, iki ayrı araştırmada tesadüf blokları deneme desenine göre 3 tekerrürlü olarak planlanmıştır. Değerlendirilen karakterlerdeki (tütünde yaş ve kuru kök ağırlığı, parazit bitkide yaş ve kuru ağırlık ve bitki sayıları) varyans analizlerine göre, özel kombinasyon yeteneği açısından tütünlerde kuru kök ağırlığı, parazit bitkilerde yaş ve kuru ağırlık ve bitki sayılarındaki farklılıklar anlamlı bulunmuştur. Bu sonuçlar, eklemeli olmayan etki gösteren genlerin önemini ortaya koymaktadır. Genel kombinasyon yeteneği önemli etkiye sahip olmadığından, ebeveynler arasında iyi ve zayıf genel kombinasyon yeteneği bulunamamıştır. Bazı hibritlerde özel kombinasyon yeteneği önemli bulunmuş ve bu nedenle şark tipi tütünlerin ıslah çalışmalarında kullanılabilecekleri tavsiyesi yapılmıştır.
Anahtar Kelimeler: Diallel, Gen etkileri, GCA, SCA, Şark Tipi Tütünler, *Orobanche aegyptiaca* Pers.

Introduction

The genus *Nicotiana* is one of the five major genera of the family Solanaceae (Goodspeed, 1954) and is divided into three sub-genera viz. *Tabacum, Rustica* and *Petunioides* mainly based on morphology, chromosomal pairing and behavior in interspecific hybrids (Siva Raju et al., 2012).

Turkish or Oriental tobacco is one of the *Tabacum* types based on morphological and biochemical criterion (Ren and Timko, 2001). This type of *Tabacum* is well known for its desirable odor and has a much milder flavor and contains less nicotine and fewer carcinogens than other varieties (Davis and Nielsen, 1999), thus is a major constituent of blend cigarette stocks (Heidari et al., 2013). It is of economic importance due to its place in worldwide crop production (8% of the tobacco world production) (Davis and Nielsen, 1999). Due to its geographical situation, northwestern Iran is one of the most favorable regions for growing Oriental tobacco (Darvishzadeh and Hatami Maleki, 2011).

Being a noxious root parasite, Egyptian broomrape (EBR) (*Orobanche aegyptiaca* Pers.), represents a major constraint in the production of Oriental tobacco and causes great losses in the yield. EBR as one of the 150 species of the genus *Orobanche* (Abbes et al., 2011) is an important holoparasitic flowering weed in the Middle East and Asia (Parker and Riches, 1993) and is one of the most important parasitic weeds in Iran (Rumsey and Jury, 1991; Hasannejad et al., 2006; Darvishzadeh et al., 2010).

EBR is an obligate parasite; it lacks chlorophyll, thus cannot synthesize its own food. To initiate its life cycle, its seeds germinate in the presence of host exudates. The seedling has to contact with a host root by its 'germ tube' or radical, (Dorr and Kollmann, 1974) immediately after germinating to survive (Abbes et al., 2011). It is by these connections that the parasite derives its nutrients and water from the host using its 'haustorium' (the tip of the elongated radicle) and establishes connections with the host vascular system (Abbes et al., 2011). Finally the part of the broomrape seedling outside the root of the host swells to form a tubercle. Under favorable conditions, a shoot bud develops on the tubercle producing a flowering spike which elongates, and emerges above the soil (Parker et al., 1993).

This parasitic weed (EBR) has a much broader spectrum of hosts and in addition to tobacco, parasitizing many other crops such as potato, tomato, faba bean, grasspea, chickpea, lentil, common vetch, cabbage, oilseed rape, carrot, peanut and several other crops (Parker and Riches, 1993; Press and Graves, 1995; Perez–de–Luque et al., 2008). Thus, eradication of this dangerous weed was and still is a worldwide aim for various reasons such as the high amount of parasite seed production, viability of seeds in the soil over several years, lack of seed germination in the absence of a chemical trigger from a suitable host, vigorous growth habit after emergence, and close association with the host crop, (Buschmann et al., 2005; Ghannam et al., 2012). Despite using a broad spectrum of control methods (mechanical, cultural, nitrogen metabolism, biological, chemical, or a combination of these (Lym, 2005; Ditomaso et al., 2006), no significant reduction of infestation has been achieved. Although host plant resistance is one of the key strategies in the fight against this parasite, because of polygenic and quantitative aspects of the resistance, there has been limited success in cultivar development with durable complete resistance. To solve this problem, the combination of conventional and molecular breeding methods (an integrated genetic improvement program) seems to be the best solution (Zhao and Chao–Chien, 2012). Preliminary information concerning the genetic makeup of a given host plant and broomrape could be obtained by conventional methods (Soliman et al., 2012). One of the several biometrical techniques available to plant breeders to achieve opening information suitable on genetic understanding for a chosen set of parents is diallel analysis (Jinks and Hayman, 1953; Jinks, 1954; Hayman, 1954a; Hayman, 1954b; Griffing, 1956a; Griffing 1956b). Little is known about the diallel of the tobacco under broomrape stress condition. The aims of the present study were to estimate the gene action and define the type of inheritance patterns of the two overall host resistance indices, broomrape flowering shoots and invaded roots on Oriental tobacco genotypes through genetic parameters.

Materials and Methods

This study was performed at Urmia Tobacco Research Centre (UTRC), with cooperation of Department of plant Breeding and biotechnology, University of Urmia, during 2012.

Parents and their hybrids

The parents used for the present study, comprised of six Oriental tobacco genotypes, G.D.165, Kromovgraid, Xanthi, SPT410, SPT406, Basmaseres31. The seeds of the fifteen intercrossed combinations (G.D.165 × Kromograid, G.D.165 × Xanthi, G.D.165 × SPT410, G.D.165 × SPT406, G.D.165 × B.S.31, Kromovgraid × Xanthi, Kromovgraid × SPT410, Kromovgraid × SPT406, Kromovgraid × B.S.31, Xanthi × SPT410, Xanthi × SPT406, Xanthi × B.S.31, SPT410 × SPT406, SPT410 × B.S.31, and SPT406 × B.S.31) of the six parents which prepared based on a half-diallel design at UTRC, were used along with their parents in this study.

Cultural practices

Seeds of the six parents and their F1 progenies were sown at a rate of approximately 5 g/m^2 followed by spreading a fine layer of sieved well fermented sheep manure on beds. Seedlings on 12 cm in height stage were transplanted to ceramic pots (one healthy and strong seedling in each pot) containing 5 kg of sterilized soil. For the experiment under broomrape condition, the soil of each pot was artificially mixed with 0.06 g Egyptian broomrape seeds. Harvests were done at technical maturity and leaves were sun-cured. The plants were not topped as is common with most other tobacco types such as Virginia and burley (Bayat et al., 2014).

Experiments

Seedlings of parents and their hybrids were used in two experiments, normal and broomrape stress conditions each was arranged in a randomized complete block design (RCBD) with three replications. Each replication consisted of one pot with one plant.

Traits measured

Several agronomic and morphological traits were used as selection criteria. For the host plants these were fresh weight of root (FWR) and dry weight of root (DWR). Dry weight percent decrease (DWPD), percent decrease in dry weight due to broomrape infestation compared with normal condition, also was used for the host plants. For parasite plants three traits namely, fresh weight of broomrape shoot (FWBS), dry weight of broomrape shoot (DWBS), and number of broomrape shoots (NBS) were used. All of these traits were measured and recorded after flowering stage for each plant per pot in each replication.

Variance and diallel analysis

The general linear model (GLM) procedure in the SAS version 9.1 software (SAS Institute Inc, NC, USA) was used for the analysis of variance. Diallel analysis were conducted according to Griffing's method 2 and model 1 (Griffing, 1956b) using the SAS program for Griffing's diallel analysis (Zhang et al., 2005). Baker ratio was used for approximate estimations of gene effects (Baker, 1978).

Results and Discussion

Analysis of variance showed significant differences among genotypes for three of investigated traits (dry weight of root, dry weight of broomrape shoot and number of broomrape shoots) under EBR stress (Table 1.). This indicated presence of adequate genetic variability among Oriental tobaccos, which could be exploited in different crossing programs. Obtaining significant differences among genotypes allowed total variation to be partitioned into variation due to general and specific combining abilities. The mean squares (MS) of GCA of the six parental genotypes and SCA of the F1 diallel crosses for the investigated traits are summarized in the Table 1. Based on significance of mean squares of SCA, non–additive gene actions were concluded. For each investigated trait after assessment of genetic parameters, parents and hybrids were investigated for their general and specific combining ability respectively.

Table 1. Variance analysis of studied traits, GCA and SCA under EBR infestation

Source of variation	df	Control		Infestation				
		FWR	DWR	FWR	DWR	FWBS	DWBS	NBS
Genotype	20	607.87	12.39	0.19	0.08*	607.87	0.21*	1114.81**
GCA	5	758.907	22.95	9.63	0.75	758.907	33.48	462.63
SCA	15	601.500	11.115	38.58	4.37*	601.500	61.51*	1223.61*
2GCA/2GCA+SCA		-	0.8	-	0.26	-	0.52	0.43

*: Significant at 5% probability level.

Dry weight of host root

Significant means of squares for SCA and Baker ratio of 0.26 showed the positive actions for non-additive effects on controlling dry weight of root (Table 1.). The best and worst general combiners were not revealed among parents for the investigated trait due to non–significance of general combining ability (GCA) (Table 2.). The best specific combination with the highest (1.57) positive SCA effects with mean root dry weight of 0.45 g/plant and dry weight percent decrease (DWPD) of 83% was Kromovgraid × SPT410 (Table 4. and Table 5.). The highest (-4.67) negative SCA effects with mean root dry weight of 0.25 g/plant and dry weight percent decrease (DWPD) of 94% was recorded in the cross GD165 × B.S.31 (Table 4. and Table 5.). Host root length and biomass reduction are escape mechanisms which could be used in resistance breeding against broomrape (Fernandez–Aparicio and Rubiales, 2010). The tobacco plant as a host of the EBR has an extensive but comparatively shallow system of fibrous roots which most of them develop adventitiously from the portion of the main stem buried during transplanting (Tso, 1999). This root system is more compatible in parasite infestation.

Thus, except in dry land farming systems where an extensive root system is essential, genotypes with suitable DWPD which does not adversely affect host plants could be considered as resistant plants based on escape mechanism.

Table 2. GCA values of studied traits under EBR infestation for the six parents

Genotypes	Control	Infestation		
	DWR	**DWR**	**DWBS**	**NBS**
G.D.165	1.67	0.14	-0.92	4.65
Kromovgraid	0.04	-0.05	1.22	5.87
Xanthi	-1.13	0.12	-0.91	-1.18
SPT410	-0.33	-0.11	1.05	-2.52
SPT406	-0.85	-0.29	-1.51	-6.57
B.S.31	0.61	0.19	1.06	-0.24

Table 3. Mean values of studied traits under EBR infestation for the six parents

Parents	Control	Infestation			
	DWR (g/plant)	**DWR (g/plant)**	**DWPD (%)**	**DWBS (g/plant)**	**NBS (n/plant)**
G.D.165	7.95	5.28	34	6.62	51.67
Kromovgraid	9.97	0.53	86	9.19	66.33
Xanthi	5.54	0.28	95	1.64	14.33
SPT410	8.66	0.43	95	1.89	8.33
SPT406	4.35	1.12	74	1.08	15.33
B.S.31	9.33	0.67	93	4.01	11.33

Dry weight of broomrape shoot per host plant

Significant means of squares for SCA and Baker ratio of 0.52 showed the positive actions for non-additive effects on controlling dry weight of broomrape shoot (Table 1.). The best and worst general combiners, were not revealed due to no difference among parents (Table 2.).

The best specific combination with highest (6.75) positive SCA effects with shoot dry weight of 0.46 g/plant was Xanthi × SPT410 (Table 4. and Table 5.). This combination could be considered as a sensitive hybrid against EBR infestation. Neither hybrid appeared as significant negative specific combination as indicative of resistant combination (Table 4.).

Number of broomrape shoots per host plant

The most widely used index among different devices for resistance to *Orobanche* is the total number of emerged shoots per host plant (Gil et al., 1987; Rubiales et al., 2006) mostly because indices based on size and weight of broomrapes can be misleading due to presence of a competition for resources among host and parasite plants (Aalders and Pieters, 1987).

Significant means of squares for SCA and Baker ratio of 0.43 showed the positive actions for non-additive effects on controlling number of broomrape shoots (Table 1.). The best and worst general combiners, were not revealed due to no difference among parents (Table 2.). The best specific combination with the highest (36.47) positive SCA effects and with the mean NBS of 30.67/plant was Xanthi × SPT410 (Table 4. and Table 5.). The worst specific combination with highest (-35.45) negative SCA effects and with the mean NBS of 17.67/plant was G.D.165 × B.S.31 followed by G.D.165 × Xanthi which showed the negative (-12.38) SCA effects with the mean NBS of 22/plant (Table 4. and Table 5.). Hybrids with negative SCA (Xanthi × SPT410 and G.D.165 × Xanthi) could be considered more resistant compared to hybrid with positive SCA (Xanthi × SPT410) in this study.

Table 4. SCA values of studied traits for the fifteen F1 hybrids

Genotypes	Control	Infestation		
	DWR	DWR	DWBS	NBS
G.D.165 × Kromovgraid	2.29	-0.79	3.86	32.58
G.D.165 × Xanthi	-0.95	-1.23	-1.51	-12.38*
G.D.165 × SPT410	1.36	-0.85	-3.23	-17.04
G.D.165 × SPT406	-2.42	0.02	-1.47	-5.99
G.D.165 × B.S.31	2.44	-4.67*	-4.59	-35.45*
Kromovgraid × Xanthi	-0.01	0.27	-2.23	-5.93
Kromovgraid × SPT410	0.73	1.57*	4.32	-2.57
Kromovgraid × SPT406	-0.84	-0.29	-0.39	-8.88
Kromovgraid × B.S.31	-2.81	-0.18	-3.79	-7.23
Xanthi × SPT410	-1.84	0.79	6.75**	36.47*
Xanthi × SPT406	-0.04	0.16	0.73	-0.15
Xanthi × B.S.31	-2.95	0.51	-2.45	-18.28
SPT410 × SPT406	-1.3	-0.29	1.67	5.52
SPT410 × B.S.31	2.77	0.33	3.76	6.73
SPT406 × B.S.31	-2.86	0.39	1.43	9.67

Table 5. Mean values of studied traits for the fifteen F1 hybrids

Hybrids	Control	Infestation			
	DWR (g/plant)	DWR (g/plant)	DWPD (%)	DWBS (g/plant)	NBS (n/plant)
G.D.165 × Kromovgraid	6.37	0.256	96	6.56	31
G.D.165 × Xanthi	4.84	0.34	93	3.07	22
G.D.165 × SPT410	6.4	0.47	93	11.61	24
G.D.165 × SPT406	4.3	0.19	96	4.31	13.67
G.D.165 × B.S.31	4.12	0.25	94	2.61	17.67
Kromovgraid × Xanthi	6.58	0.32	95	2.53	21
Kromovgraid × SPT410	2.67	0.45	83	11.89	56
Kromovgraid × SPT406	3.92	0.32	92	3.31	15.33
Kromovgraid × B.S.31	5.37	0.43	92	2.05	3.67
Xanthi × SPT410	4.42	0.05	99	0.46	30.67
Xanthi × SPT406	3.47	0.17	95	6.21	19.67
Xanthi × B.S.31	8.14	0.25	97	4.24	12.67
SPT410 × SPT406	7.99	0.22	97	0.97	10
SPT410 × B.S.31	6.59	0.30	95	4.99	26
SPT406 × B.S.31	5.58	0.52	91	18.45	66.67

Conclusions

Partitioning of total mean squares of genotypes to general and specific combining abilities revealed non–additive gene effects for investigated traits under EBR. Two out of fifteen hybrids appeared to be positive significant SCA. These were Kromovgraid × SPT 410 for root dry weight of host (1.57) and Xanthi × SPT 410 for dry weight of broomrape shoot (6.757) and number of broomrape shoots (36.47). Hybrid G.D.165× B.S.31 showed negative significant SCA both for root dry weight of host and number of invaded shoots (Table 6.).

Table 6. Significant SCAs of studied traits under EBR stress

SCA	Positive			Negative	
Trait	DWR	DWBS	NBS	DWR	NBS
Hybrid	2×4	3×4	3×4	1×6	1×6
Significant SCA	1.57*	6.757**	36.47*	-4.67*	-35.45*
GCAs of respective parents	-0.05×-0.11	-0.91×1.05	-1.18×-2.52	0.14×0.19	4.65×-0.24

*: Significant at 5% probability level, ns: Non-significant, 1: G.D.165, 2: Kromovgraid, 3: Xanthi, 4: SPT 410, 5: SPT 406, 6: B.S.31, A: Hybrid, , B: Significant SCA, C: GCAs of respective parents, 1: Gene action mode : Additive & Non–additive (Non–additive>Additive).

Acknowledgements: The authors wish to thank the Urmia Tobacco Research Centre (UTRC), Iran for providing genetic materials and facility for conducting research work.

References

Aalders, A.J.G., Pieters, R., 1987. Resistance in *Vicia faba* to Orobanche crenata: true resistance versus hidden susceptibility. Euphytica. 36: 227–236.

Abbes, Z., Kharrat, M., Djebali, W., Haibi, C., 2011. Interaction between Broomrapes and their hosts. J. Phytol. 3 (1): 68–72.

Bayat, M., Darvishzadeh, R., Soleimani, F., Alavi, S.R., 2014. Sequentialz path analysis for determining interrelationships between yield and related traits in tobacc(*Nicotiana tabacum* L.) under normal and abiotic stress conditions. Genetika. 46 (3): 815–829.

Baker, R.J., 1978. Issues in diallel analysis. Crop Sci. 18: 533–536.

Buschmann, H., Gonsior, G., Sauerborn, J., 2005. Pathogenicity of branched broomrape (*Orobanche ramosa*) populations on tobacco cultivars. Plant Pathol. 54: 650–656.

Darvishzadeh, R., Alavi, R., Sarrafi, A., 2010. Resistance to powdery mildew (Erysiphe cichoracearum DC.) in oriental and semi–oriental tobacco germplasm under field conditions. J. Crop Improv. 24: 122–130.

Darvishzadeh, R., Hatami Maleki, H., 2011. Analysis of genetic variation for morphological and agronomic traits in Iranian oriental tobacco (*Nicotiana tabacum* L.) genotypes. Crop Breed. J. 2 (1): 57–61.

Davis, L., Nielsen, M., 1999. Tobacco: Production, chemistry and technology. Oxford, UK: Blackwell Science.

Ditomaso, J.M., Kyser, G.B., Miller, J.R., Garcia, S., Smith, R.F., Nader, G., Connor, J.M., Orloff, S.B., 2006. Integrating prescribed burning and clopyralid for the management of yellow starthistle (Centaurea solstitialis), Weed Sci. 54: 757–767.

Dorr, I., Kollmann, R., 1974. Structural features of parasitism of *Orobanche*. I. Growth of the haustorial cells within the host tissue. Protoplasma. 80: 245–249.

Fernandez–Aparicio, M., Rubiales, D., 2010. Characterisation of resistance to crenate broomrape (Orobanche crenata Forsk.) in *Lathyrus cicera* L. Euphytica. 133: 77–84.

Ghannam, I., Al–Masri, M., Barakat, R., 2012. The Effect of Herbicides on the Egyptian Broomrape (*Orobanche aegyptiaca*) in Tomato Fields. American J. Plant Sci. 3: 346–352.

Gil, J., Martı´n, L.M., Cubero, J.I., 1987. Genetics of resistance in V. sativa to O. crenata Forsk. Plant Breed. 99: 134–143.

Goodspeed, T.H., 1954. The genus *Nicotiana*. Waltham, Mass., USA: Chronica Botanica.

Griffing, B., 1956a. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10: 31–51.

Griffing, B., 1956b. Concepts of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9: 463–493.

Hasannejad, S., Zad, S.J., Alizadeh, H.M., Rahymian, H., 2006. The effects of *Fusarium oxysporum* on broomrape (*Orobanche aegyptiaca*) seed germination. Commun. Agric. Appl. Biol. Sci. 71: 1295–1299.

Hayman, B.I., 1954a. The analysis of variance of diallel tables. Biometrics. 10: 235–244.

Hayman, B.I. 1954b. The theory and analysis of diallel crosses. Genetics. 39: 789–809.

Heidari, A., Darvishzadeh, R., Fayyaz Moghaddam, A., Rastgou, M., Hatami Maleki, H., 2013. Investigation on the response of oriental and semi oriental tobacco genotypeto Potato Virus Y under controlled conditions. Iranian J. Genet. Plant Breed. 2 (1): 30–38.

Jinks, J.L., 1954. The analysis of continuous variation in diallel crosses in *Nicotiana rustica* L., varieties. Genetics. 39: 367–788.

Jinks, J.L., Hayman, B.I., 1953. The analysis of diallel crosses. Maize Genet. Coop. News Lett. 27: 48–54.

Lym, R.G., 2005. Integration of biological control agents with other weed management technologies: Successes from the leafy spurge (Euphorbia esula) IPM program, Biol. Control. 35: 366–375.

Parker, C., Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. Wallingford, UK: CAB International.

Press, M.C., Graves, J.D., 1995. Parasitic Plants. London, UK: Chapman & Hall.

Pérez–de–Luque, A., Moreno, M.T., Rubiales, D., 2008. Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance. Ann. Appl. Biol. 152: 131–141.

Pérez–de–Luque, A., Fondevilla, S., Pérez–Vich, B., Aly, R., Thoiron, S., Simier, P., Castillejo, M.A., Fernandez, J.M., Jorrin, J., Rubiales, D., Delavault, P., 2009. Understanding broomrape host plant interaction and developing resistance. Weed Res. 49: 8–22.

Ren, N., Timko, M.P., 2001. AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild *Nicotiana* species. Genome. 44: 559–571.

Rubiales, D., Pérez–De–Luque, A., Fernandez–Aparicio, M., 2006. Screening techniques and sources of resistance against parasitic weeds in grain legumes. Euphytica. 147: 187–199.

Rumsey, J., Jury, S.L., 1991. An account of Orobanche L. Britain and Ireland. Watsonia. 18: 257–295.

Siva Raju, K., Sharma, R.K., Murthy, T.G.K, 2012. Genetic and Evolutionary Relationship among *Nicotiana* species as elucidated by AFLP. J. Agric. Sci. 7 (3): 135–144.

Soliman, M.M., Abdallah, N.G., Bakheit, M.A., Raslan, M.A., Abd–El–Haleem, S.H.M., 2012. Directional Selection in Faba Bean (*Vicia faba* L.) Under Infestation of *Orobanche crenata*. World Appl. Sci. J. 16 (8): 1074–1081.

Tso, T.C., 1999. Seed to smoke In: Davis, L., and M. Nielsen (eds). Tobacco: Production, chemistry and technology. Oxford, UK: Blackwell Science.

Zhang, Y., Kang, M.S., Lamkey, K.R., 2005. Diallel–SAS05: A comprehensive program for Griffing's and Gardner–Eberhart analyses. Agron. J. 97: 1097–110.

Zhao, L., Chao–Chien, J., 2012. Molecular techniques for sunflower breeding. In: Skoric, D., Seiler, G.J., Zhao, L., Chao–Chien, J., Miller, J.F., Charlet, L.D. Sunflower genetics and breeding. Int. Monography. Serbian Acad. Sci. Arts, Branch in Novi Sad: 520.