



## Genetic Variability and Resistance to *Orobanche* in Oriental *Nicotiana tabacum* L.

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### ABSTRACT

Broomrape as an obligate parasite that threatens tobacco production (*Nicotiana tabacum* L.), has destructive effects on its characteristics. The reduction in yield caused by this parasite is so great that sometimes farmers are forced to leave their fields due to the severity of the infection. Since the stages of contamination and pathogenicity mainly occur underground, and the major damage of the parasite to host plants produce before the diagnosis of contamination, the development of effective controlling strategies is a great challenge in front of scientists. Unfortunately, no control method (agricultural, chemical, mechanical, etc.) has been effective so far. The lack of effective agro-chemical controlling methods makes the need more obvious for biotechnological methods. Host resistance as an integrated pest management method is a multifaceted process that can occur at several stages of the parasite's life cycle; before attaching to the host during root penetration, or after attaching to the phloem. In recent years, various breeding activities have been started to develop "*Orobanche*-resistant" genotypes. In this paper, the conducted research activities on the genetic variability and resistance of tobacco to broomrape have been reviewed. The results determine the importance and also the path to achieving "*Orobanche*-resistant" varieties.

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### 1. Introduction

Broomrape (*Orobanche*) belongs to the family Orobanchaceae with annual as well as perennial life forms. This parasite plant could attack the root part of a vast range of dicot plants and infect them (Roman *et al.*, 2003; Schneeweiss *et al.*, 2004). *Gymnocaulis* Nutt., *Myzorrhiza* (Phil.) Beck, *Trionychon* Wallr., and *Orobanche* (syn. *Osproleon* Wallr.) (Román *et al.*, 2003) are the sections that constitute this genus. The *Trionychon* and *Orobanche* sections of the above-mentioned items are notable for containing the famous broomrape species. For example, *O. ramosa* L. and *O. aegyptiaca* belong to the section *Trionychon* (Paran *et al.*, 1997). Cytogenetic studies (Schneeweiss *et al.*, 2004) revealed the existence of three basic chromosome numbers for the genus *Orobanche* including  $x = 19$  in section *Orobanche*,  $x = 12$  in *Myzorrhiza* and *Trionychon* and  $x = 24$  in *Gymnocaulis*. A literature review (Musselman, 1980)

showed the presence of 150 species within the genus *Orobanche* of which *O. aegyptiaca* and *O. cernua* are momentous regarding their vast host range (Brassicaceae and Fabaceae) and their capacity to infect the majority of crops in family Solanaceous such as potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.) (Fig. 1), and tomato (*Lycopersicon esculentum* Mill.). Likewise, *O. crenata* is another known specie of *Orobanche* that could infect feeding crops such as *Lathyrus sativus* L., *L. cicera* L., *Vicia sativa* L., and *V. villosa* L. (Linke *et al.*, 1993). *O. cumana* is the next species most commonly found in sunflower fields (Pineda-Martos *et al.*, 2014).

Due to the lack of chlorophyll and very low transpiration rate, the parasite obtains the nutrients it needs from the host's phloem through the process of osmotic regulation with the accumulation of potassium and soluble sugars at a high level (Abbes *et al.*, 2009). In fact, after attaching to the vascular system of the host

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plant through the haustorium, the parasite acts as a strong reservoir for photosynthetic materials of crops and restricts their growth (Fernández-Aparicio *et al.*, 2016).



Figure 1. Tobacco field infected by *Orobanche*.

Sucrose is the most important compound derived by the parasite from the host plants, and it is broken down in the tuber. Sucrose decomposition has been associated with the accumulation of hexoses and to a less extent mannitol and starch in the tuber of *O. ramosa* (Abbes *et al.*, 2009; Delavault *et al.*, 2002). In other parasitic weeds such as *Striga* (Stewart *et al.*, 1984) and *Thesium* (Fer *et al.*, 1994; Simier *et al.*, 1994), hexoses and mannitol are also important osmotic agents. An important gene in the biosynthesis of mannitol by parasites is mannose 6-phosphate reductase (M6PR). The study of changes in the activity of this enzyme and the content of mannitol in different developmental stages of *O. ramosa* has shown that the accumulation of hexoses and mannitol increases in the parasite, especially in branches after its binding to host plants (Delavault *et al.*, 2002). Another enzyme involved in many source-reservoir relationships and creating higher osmotic pressure is invertase; responsible for converting sucrose to glucose and fructose. Investigation of nitrogen and carbon relationships between *O. foetida* and sensitive and resistant bean lines showed when the parasite attaches to the roots of tolerant lines, the activity of soluble invertases is limited in the tubers especially in the branches of the parasite which indicates a decrease in the parasite capacity for using host carbohydrates and a lower sink power (Abbes *et al.*, 2009). The study of sucrose synthase (SUS), another key enzyme involved in sucrose degradation, showed the highest level of accumulation of this enzyme in *O. ramosa* tuber when the parasite is attached to the tomato vascular system (Péron *et al.*, 2012). The importance of these genes

(PaM6PR, Pacwi, and Pasus1) has been proven in the osmotic regulation of different parasite organs at different developmental stages of two important species of *O. ramosa* and *O. aegyptiaca* (Farrokhi *et al.*, 2019a).

Annual yield decline due to *Orobanche* is estimated about \$1.3 billion to \$2.6 billion just in the Middle East (Aly, 2007). The widespread damage of *Orobanche*, its uncontrollability, as well as the tubers produced by the parasite on important crops, have caused farmers to call this parasitic weed cancer. The decrease in yield caused by the parasite is so great that sometimes farmers are forced to leave their fields due to the severity of the infection.

Tobacco is considered an industrial and medicinal plant that is cultivated in more than 100 countries and about 4.2 million hectares of agricultural land (Davalieva *et al.*, 2010). Albeit, leaves are the commercial portion of the plant, tobacco seeds comprise 38% of nonedible oil and therefore it could be a suitable candidate for diesel fuel (Giannelos *et al.*, 2002). Broomrape infection was observed in tobacco fields, and several methods including field practices and chemical control have been developed to control the spread of the weed (Zwanenburg *et al.*, 2016; Rubiales *et al.*, 2009). Regarding broomrape characteristics such as its underground connection with the host and numerous small seeds (Buschmann *et al.*, 2005), the chemical as well as field practices were not successful and the development of resistant varieties will be more effective (Pérez-de-Luque *et al.*, 2008). The use of resistant cultivars is an environmentally friendly and economical method that has significant durability from an ecological point of view (Slavov *et al.*, 2005).

## 2. Genetic variability in *Orobanche* population

In general, *Orobanche* must evolve to overcome host resistance over time. Host-parasite co-evolution is a mutual selection in nature on host resistance and parasite contamination (Hu *et al.*, 2020). So, The screening and evaluation of host germplasm and parasite organisms is an ongoing responsibility that plants disease resistance breeders must prioritize (Buschmann *et al.*, 2005). Regarding the genetic diversity of *Orobanche*, there is a comprehensive work carried out using ISSR markers to inspect the genetic variability among 44 *Orobanche aegyptiaca* specimens

sampled from northwest Iran (Abedi *et al.*, 2014). In this research, Abedi *et al.* (2014) found 261 loci using 20 ISSR markers and the majority of them (94%) were polymorphs. They obtained significant diversity genetic among the studied samples and separated the examined *Orobanche* into six groups using the UPGMA algorithm. In the reported results (Abedi *et al.*, 2014), the Jacquard similarity coefficients varied from 0.71 (between individuals 23 and 27) to 0.34 (between 13 and 30). Population structure analysis using the Bayesian method also was studied (Abedi *et al.*, 2014) and consequently, 44 *Orobanche* samples were located in two subpopulations and the remained samples were identified with admixture genomes regarding their Q values. Finally, most of the genetic variation (99%) was found within *Orobanche* groups via AMOVA and so, notification to *Orobanche* genetic variability is unavoidable in tobacco breeding for tolerance/resistance to *Orobanche*.

### 3. Sources of resistance to *Orobanche*

As mentioned earlier, host resistance is increasingly considered an important method to achieve effective integrated pest management (Goldwasser *et al.*, 1999). In a previous report (Parker and Riches, 1993), Significant diversity for resistance against broomrape was reported in tobacco germplasm. In a study, Darvishzadeh (2016) evaluated the reaction of 89 oriental and water pipe tobacco genotypes against *Orobanche* in RCBD design with three blocks during two consecutive years. In each environment (year), the tobacco varieties were planted in non-inoculated and inoculated conditions, where the soil of pots was mixed with 0.06 g of *Orobanche* seed. In biotic stress conditions, the number of emerged broomrapes (Fig. 2) varied between 0.0 to 5.0. The fresh weight of broomrapes ranged between 0.0 and 71.6 g and the dried weight of broomrapes fluctuated from 0 to 23.1 g. Darvishzadeh (2016) found that tobacco genotypes 'G.D.165', 'Krumovgraid', 'Ohdaruma', 'Ts8', 'HTI', 'C.H.T. 209.12e', 'L17', and 'C.H.T. 266-6' out of studied genotypes had the minimum value of the number of emerged broomrapes (below 0.5) and genotypes 'T.B.22' and 'Kramograd N.H.H. 659' had no infection to *Orobanche*. The maximum infection to

*Orobanche* was detected in genotype 'Borazjan' (a water pipe's tobacco type).

In the other study (Tahmasbali *et al.*, 2020a), the resistance/susceptible reaction of 92 oriental-type tobacco along with water pipe's tobacco genotypes was inspected under normal and *Orobanche* infected conditions at Urmia Tobacco Research Center of Iran. Interestingly, a high genotypic coefficient of variation ( $CV_g$ ) was seen for interested traits (flowering date, plant height, number of leaves, surface of leaf, fresh weight of leaf, dry weight of leaf, fresh weight of root, dry weight of root, fresh weight of plant, dry weight of plant), especially for the fresh weight of leaf (FWL; gr) and dry leaves yield per plant (DLYP; gr) in normal and *Orobanche* infected conditions. In each environment, the studied tobacco germplasm was classified into four distinct groups. In *Orobanche* infected state, rankings of the groups based on traits average showed that the second and fourth groups comprised genotypes that are resistant and sensitive to *Orobanche* infection, respectively.

Afterward, regarding dry leaves yield per plant (DLYP; gr) in both normal (Yp) and *Orobanche* infection conditions (Ys), nine stress tolerance indices comprising tolerance index (TOL), relative stress index (RSI), mean productivity (MP), harmonic mean (HM), yield stability index (YSI), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI) and yield index (YI) were calculated (Tahmasbali *et al.*, 2021b). Results showed that MP, HM, GMP, STI, and YI indices had positive significant relation with yield in stress (Ys) and non-stress (Yp) conditions. Using biplot analysis, tobacco genotypes 'C.H.T.209.12e×F.K.40-1', 'C.H.T.209.12e', 'H.T.I', 'F.K.40-1' and 'Krumovgraid' were identified as high yielding and tolerant genotypes and genotypes 'SPT 430', 'SPT 420', 'SPT 433', 'SPT 412', 'SPT 413', 'SPT 432' and 'Matianus' as the most sensitive genotypes to *Orobanche* stress. Typically, the genetic diversity in the studied tobacco germplasm in response to *Orobanche* infection can be used in selecting suitable parents for breeding programs, creating segregating populations to identify genomic regions that control *Orobanche* tolerance, and studying changes in the expression of tolerant genes.





Figure 2. Pots washing steps in order to count the number of *Orobanche*.

#### 4. Genetic analysis of resistance to broomrape

##### 4.1. Estimation of breeding value (BV)

A critical step in hybrid breeding programs of interested plants is parental selection. In this regard, various methods have been implemented to identify promising genotypes suitable for the target agronomical traits including *Orobanch* tolerance. Predicted breeding values (White and Hodge, 1988) numerously are used for parental selection in animal breeding. Two common methods for predicting the breeding value of parents are the selection index and best linear unbiased prediction (BLUP). About BLUP, Tahmasbali et al. (2020b) predicted the breeding value of several agro-morphological traits in both normal and *Orobanche* stress conditions. Accordingly, genotype C.H.T.209.12e 12 F.K.40-1 possessed high yield under both conditions and was considered as appropriate genotypes for crossing programs. As a result, genotype 'Rustica' had the best breeding value regarding studied characters in both conditions. It is concluded that a genotype with good phenotypic performance may have low breeding value and so, in tobacco breeding programs, breeding value information accompanied by the mean of phenotypic characters must be considered.

In the other study (Tahmasbali et al., 2021a), selection indices such as optimal selection (Smith-Hazel), Base (Brim), desired gain (Pesek-Baker) and Robinson were calculated for tobacco agro-morphological data obtained in normal and *Orobanche*

stress states. The findings showed that genotypes with more value for leaf area, fresh weight of leaf, and fresh weight of aerial parts have potentially higher performance. Also, Smith-Hazel and Brim indices with the highest selection efficiency (DH) in both states will result in increasing the leaf area and fresh weight of leaf in normal conditions and leaf area and fresh weight of aerial parts in *Orobanche* stress conditions. On the other hand, the fresh weight of the leaf and fresh weight of aerial parts were the traits with the highest correlated response with the dry weight of the leaf, therefore, these two indices with the highest correlation with breeding value and with the highest relative efficiency of selection are presented as the best indices. Among studied germplasm, the genotype H.T.I was recognized as the suitable genotype under normal and *Orobanche* stress conditions.

##### 4.2. Estimation of GCA and SCA

Selection of *Orobanche* tolerant tobacco lines would be feasible only if the observed genotypic differences are heritable. Seyyed-Nazari et al. (2016) investigated the genetic basis of variability for the fresh weight of leaf (FWL), dry weight of leaf (DWL), number of leaves (NL), length of leaf (LL), the width of the leaf (WL), length of stem (LS), and diameter of the stem (DS) in tobacco under normal (without *Orobanche*) and stress (with *Orobanche*) conditions. Genetic designs such as Griffing's diallel method proved the

existence of GCA and SCA for agro-morphological attributes of tobacco except for the length of the leaf and therefore, both additive and non-additive effects are responsible for controlling these characteristics. Baker ratios as another measured parameter showed the magnitude of additive genetic effect in inspected characters, except for the number of leaves that both components had equal importance. Regarding data on broomrape (Seyyed-Nazari *et al.*, 2015), predominant non-additive genetic effects were detected for characters including the 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) by diallel analysis. In the end, the identified hybrids with principal SCA could be utilized in oriental-type tobacco breeding schedules.

#### 4.3. QTL mapping

To our knowledge, the majority of economic attributes of plants (also under stress state) are controlled by multiple genes that are affected by environmental factors. So, with the appearance of DNA markers, it is possible to determine genomic regions controlling such characters and do important steps in their breeding programs. In a study, Darvishzadeh (2016) identified five SSR loci distributed across several linkage groups (2, 10, 11 and 18) of tobacco reference map (Bindler *et al.*, 2007, 2011) with significant relation with genomic regions controlling *Orobanche* resistance in tobacco. Using an association mapping approach through a mixed linear method (Tahmasbali *et al.*, 2022) a total of 16 SSR loci were identified with significant roles ( $P < 0.05$ ) in controlling tobacco agro-morphological characters under normal (without *Orobanche*) and stress (with *Orobanche*) conditions. Some common markers were identified for a particular trait across normal and *Orobanche* conditions.

### 5. Breeding for *Orobanche* resistance

#### 5.1. Conventional and molecular approach

In breeding programs, the success of selection importantly depends on the presence of genetic diversity of interested characters. Regarding the relatively small genetic base of cultivated tobacco, the existing genetic resources are an invaluable source of variability, which can be used for the introduction of

agro-morphologically important genes to improve the quality and economic value of tobacco crops. Wild tobacco species and indigenous varieties have proven to be crucial in providing resistance to broomrape and have also been utilized to introduce resistance against it (Pineda-Martos *et al.*, 2013).

Marker-assisted selection (MAS) offers a simple, more efficient, accurate breeding method, suitable for breeding to disease resistance matched with selection based on phenotype (Hasan *et al.*, 2021). As broomrape resistance is introduced into cultivated tobacco from various sources, the origin of the material is an important factor when it comes to the mode of inheritance and the position of resistance genes. Different types of molecular markers have been employed for the localization and identification of region(s) carrying resistance genes. With the appearance of more intricate, quantitative broomrape resistance in tobacco, analyzing and recognizing genes for resistance has become more puzzling, and requires the use of more accurate tools.

#### 5.2. Transgenic approach

Creating transgenic plants is a promising and cost-effective alternative method to chemical ones. This method reduces labor and costs, increases crop choices, and eliminates the need for chemicals that may be harmful to the environment. New strategies for engineered host plant resistance to parasites include the expression of parasite-specific toxins in host roots and RNAi for silencing genes vital for parasite development. RNA interference (RNAi) is a natural regulatory mechanism in the growth, development, and host defense against viruses, transposons, and its role as a powerful strategy for engineering resistance against diseases (viruses, nematodes, insects, and fungi) and parasitic weeds in plants have been proven (Aly *et al.*, 2009; Dubey *et al.*, 2017; Farrokhi *et al.*, 2019b). A highly efficient way to deliver resistance to the host by gene silencing is to transform plants with transgenes expressing hairpin structures and capable of forming dsRNA (Morozov *et al.*, 2019). The advantages of using this method include a high degree of immunity (not engaging with DNA and reducing mutations), high efficiency due to suppression of gene expression, and specificity of gene silencing (due to accurately identifying the target gene). If this structure is designed from parasite-specific sequences, even

when dsRNA is converted to siRNAs, no phenotypic sign will appear in the host, but the transmission of the RNAi silencing signal to the parasite through the haustorium prevents the expression of the gene and thus parasite survival (Aly *et al.*, 2009). Intercellular transmission of signals, including RNA, usually occurs through plasmodesmata and in the long-distance transmission pathway through the vascular system, especially the phloem, which provides a symplastic connection between plant organs (Aly, 2007). SiRNA delivery systems use a variety of methods to transport these molecules into cells. The most important methods of transmission are stable genetic transmission by Agrobacterial tDNA, electroporation, cell injection by microinjection and delivery using nanotechnology-based delivery systems.

Transient silencing of *O. aegyptiaca* genes by injection of quenching structures into the tomato host caused significant reductions in gene expression, enzyme activity, and sugar amounts (Farrokhi *et al.*, 2019b). Production of transgenic plants expressing the dsRNA construct against the M6PR gene by Aly *et al.* (2009) showed a significant reduction in the mRNA level of this gene and the total amount of mannitol in the parasitic tuber of the broomrape parasite. In addition, the researchers reported a 20-fold increase in parasite tuber mortality in transgenic tomato plants compared to non-transgenic lines (Aly *et al.*, 2009). In the study of Dubey *et al.* (2017), three important genes involved in the metabolism of *O. aegyptiaca* parasite (PaACS, PaM6PR, and PaPrx1) were selected to test via gene silencing strategy. Using the VIGS system, only two genes, PaASC and PaM6PR, were silenced, but in the stable transgenic system, with the help of hairpin structures, PaPrx1 transcripts, in addition to the previous two genes, were significantly reduced in parasitic tuber attached to the roots of transgenic plant lines. Lack of decreased PaPrx1 expression in the transient transmission system may be due to instability or inefficiency of TRP-derived PaPrx1-siRNAs or due to siRNA localization in tissue (Dubey *et al.*, 2017).

According to the investigations, there exists a multitude of methods and strategies that may be employed to achieve enduring broomrape resistance in tobacco. Nonetheless, the solution does not reside in a singular methodology, but in the combination of multiple approaches and the development of statistical and genetic tools for appropriate exploitation. Based on

the findings gleaned from current research data, the optimal approach for mitigating the deleterious effects precipitated by broomrape entails the development of resistant tobacco cultivars capable of enduring Orobanche's variegated strains. Numerous imperative objectives ought to be established in the forthcoming period. In order to address the issue at hand, it is imperative to conduct a comprehensive identification of all the extant tobacco broomrape varieties using a uniform set of differential lines across the entire nation. Additionally, it is essential to ascertain the composition map and geographical distribution of the broomrape race category. Secondly, there appears to be utility in the exchange of resistant breeding materials with tobacco breeding teams located internationally to generate tobacco varieties resistance. Thirdly, there is a pressing need for molecular research to investigate the intricate interaction between broomrape and tobacco to explore the mechanism of broomrape pathogenesis as well as resistance in tobacco. In order to effectively manage broomrape infestations in tobacco crops, it is recommended that a comprehensive set of integrated control techniques be organized and implemented. Consequently, the effective management of Orobanche in tobacco is a consequence of collaborative efforts undertaken by pre-breeding specialists, breeders, plant pathologists and biotechnologists.

### Conflict of Interests

All authors declare no conflict of interest.

### Ethics approval and consent to participate

No human or animals were used in the present research.

### Consent for publications

All authors read and approved the final manuscript for publication.

### Availability of data and material

All the data are embedded in the manuscript.

### Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

### Informed Consent



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