Breeding for Ascochyta blight resistant, high-quality kabuli chickpea varieties in Bulgaria

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(Manuscript received 7 April 2009; accepted for publication 9 April 2009)

Abstract. *Chickpea cultivation in Bulgaria is limited by susceptibility to the devastating disease ascochyta blight (caused by Ascochyta rabiei). The most economical and effective means of controlling damages is the creation of resistant varieties. The goal of this investigation was: (i.) to analyze the frequency and severity of Ascochyta blight of chickpea in Bulgaria; and (ii.) to develop high quality and productive chickpea varieties with resistance to the disease. The investigation was carried out at Dobrudzha Agricultural Institute, General Toshevo from 1982-2008. Over 14,200 Bulgarian and foreign varieties, germplasm accessions, breeding populations and hybrid and mutant progenies, were analyzed mainly for ascochyta blight resistance and suitable agronomic characteristics (e.g. phenology, plant morphology, seed size and colour). Blight resistance and yield was determined in field nurseries. The area of the plots was 10 m^2, in 4 replications. Ascochyta infested chickpea plant debris was collected every year and used to inoculate the field nurseries in the following year. The disease rating scale developed at ICARDA was used to score the plant material where 0=no disease infection and 9=all plants dead. The results of international collaboration and applied breeding methods – hybridization and mutagenesis, led to the development of two registered chickpea varieties – 'Balkan'and 'Progres' which have high production potential, good to very good field resistance to ascochyta blight, and good seed quality traits. Many hybrid and mutant lines with very good productive potential and blight resistance are currently being evaluated in the breeding nursery.*

Key words: ascochyta blight, chickpea, variety, resistance

Introduction

Chickpea is one of the world's most important grain legume: in 2003 the world production reached 7.1 million MG, ranking third behind dry bean (*Phaseolus vulgaris* L.), and dry pea (*Pisum sativum* L.) (FAO, 2004). Chickpea is an old and traditional crop for Bulgarian agriculture and has been utilized as a fodder and food since the 12th century. The area planted with chickpea in the country has always been small and has fluctuated - from 20,000 ha in 1945 to 3-4,000 ha in recent years. Average yields range from 800-1000 kg/ha. With the development and adoption of new varieties combined with improved production technology, many farmers have had yields ranging from 2000 to 3000 kg/ha.

The major limiting factor in chickpea production is susceptibility to the most economically important disease Ascochyta blight (caused by *Ascochyta rabiei* [Pass] Labr.). The disease causes low yields and unstable production from **dob.atanasova@abv.bg*

year to year. Yield losses in years with disease epiphytotics and susceptible varieties have ranged from 50% (Kovatchevski, 1936) to 100% in the following years: 1981, 1988, 1991, 1993, 1998, 1999, 2000, 2004 and 2006 (Nene and Reddy 1987; Kaiser, 1992; Acikgoz et al., 1994; Stanoeva, 2000, 2003; Mihov at al., 2005; Atanasova and Mihov, 2006b; Stanoeva, 2006).

The most certain tool to overcome ascochyta blight is to create new and more productive varieties with good field resistance (Bretag et al., 2003; Mihov at al., 2005; Atanasova and Mihov, 2006; Stanoeva, 2006). Breeding for disease resistance is a complicated, difficult and prolonged process requiring the combined efforts of many specialists. It requires also looking for new resistance sources, methods of work and assessment of relatively high amount of breeding materials in laboratory and field conditions. The difficulty is also determined by the pathogene ethiology - the presence of a sex reproductive form which contributes to the genetic diversity in the pathogen population. Diversity in the pathogen population has been responsible for the increased susceptibility of many varieties (Kaiser 1992; Stanoeva and Kiryakov 2003; Mihov at al., 2005).

Chickpea is a productive legume crop with high protein content. It has good resistance to drought and is suitable for mechanized production and harvesting. Under Bulgarian conditions, chickpea has higher yields when compared to pea, bean, lentil and soya in regions of the country that are prone to drought. The crop is not attacked by granivore. The root system develops numerous nodules that enrich the soil with nitrogen as the result of symbiosis with *Rhizobium ciceri*. The crop matures and is harvested relatively early in the growing season. It is an excellent predecessor for winter cereals crops.

Chickpea varieties resistant to ascochyta blight were needed for consistent production of the crop. The goal of this investigation was: (i.) to analyze the frequency and severity of Ascochyta blight of chickpea in Bulgaria; and (ii.) to develop high quality and productive chickpea varieties with resistance to the disease.

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Materials and methods

The investigation was carried out at Dobrudzha Agricultural Institute, General Toshevo from 1982-2008. Bulgarian and foreign varieties, germplasm accessions, breeding populations, hybrid and mutant progenies, were analyzed mainly for ascochyta blight resistance and suitable agronomic characteristics (e.g. phenology, plant morphology, seed size and colour). Blight resistance and yield was determined in field nurseries. The agronomic assessment of varieties and promising lines was done in competitive variety trials using the randomized complete block method with four replications. Each individual plot was planted in a 10 m^2 area. Sowing rate was 50 germinating seeds/m^2. Ascochyta infested chickpea plant debris was collected every year and used to inoculate the field nurseries in the following year. The disease rating scale developed at ICARDA was used to score the plant material where 0=no disease infection and 9=all plants dead.

Bulgarian and foreign varieties and lines, mainly from ICARDA, Syria; USDA-ARS Grain Legume Project at Pullman, USA; The Vavilov Institute, St. Petersburg, Russia and several other sources were used in intervarietal hybridizations. For wide hybridizations were used annual species *C. ehinospermum* and *C. reticulatum*, and also an attempt was made to use the perannual wild species - *C. anatolicum* and *C. montbretii*. These sources of germplasm were crossed with productive cultivated varieties and lines. Mutagenic treatments included different doses γ-rays Co^{60} and EMS either alone or in combination. The most frequently used breeding method for the hybrid and mutant populations was individual plant selection and the pedigree method, with some modification. In all cases, elite plants were selected with desired characters and evaluated in succeeding generations.

Results and Discussion

Considerable amount of initial materials have been collected during expeditions within the country aided by international collaboration with ICARDA, Aleppo, Syria; USDA-ARS, Pullman, USA; Vavilov Institute, St. Peterburg, Russia and other collaborations. At this time methods of hybridization and experimental mutagenesis were established and used (Table 1). The large amount of initial material enabled the registration of two new highly productive varieties with good field resistance to ascochyta blight.

Table 1. The origin of the initial germplasm used to breed chickpea at Dobrudzha Agricultural Institute from 1982 to 2008.

Origin of chickpea breeding material	Number	%
Materials from the country – varieties, hybrid and mutant progenies and lines	6877	48.00
Materials from ICARDA – hybrid populations and selected elite plants – progenies and lines	4830	33.80
Materials from USDA-ARS, Pullman	562	3 90
Materials from VIR, Russia and other countries	88	0.62
Materials include in variety testing trials	1927	13.50
All analyzed materials	14284	100.00
Approved new varieties	2	

In order to determine the frequency and the level of infection of the ascochyta blight the reactions of the susceptible varieties, Resursi 1 and ILC 262 were observed (Fig. 1).

In 14 of the 27 years of evaluations, the susceptible varieties showed reaction scores of 7-9, in 8 years they showed a low level of infection with scores of 1-3 and in 5 years blight did not develop and the lines were scored as 0. This analysis indicates that ascochyta blight is a detrimental and limiting factor in chickpea production in most years in Bulgaria.

During the past 25-30 years, hybridization and experimental mutagenesis has been used as a tool to develop improved varieties and to create new genetic diversity in chickpea. As a result some basic knowledge concerning genetics and breeding of the crop has accumulated (Atanasova and Mihov, 2006). Over 250 hybrid combinations had been made in intervarietal hybridization. Concerning distant hybridization 87 successful hybrids were made using *C. echinospermum* and *C. reticulatum*; however, we have not had success in making crosses between cultivated chickpea and any of the perennial species. In experimental mutagenesis, different varieties and promising lines were treated with γ-rays and chemical mutagens at different doses, alone and in combination (Atanasova and Mihov, 2006). Thanks to the frequent manifestation of ascochyta blight in the field, there were possibilities to select new lines with partial resistance. The first lines with blight resistance were selected from germplasm from ICARDA and later from our hybrid and mutant populations. The resistance to blight was also studied in young phases of the plants using selected lines.

Field reactions to ascochyta blight of the new varieties, candidate-varieties and promising lines during 2004, 2006 and 2008 are shown in Table 2. Almost all of the lines showed resistance on different levels compared to the susceptible variety Resursi 1. The best field and laboratory resistance was shown by FLIP 95-44c-32231, FLIP 95-58c1-2-4, FLIP 92-155c-1-1-7, X97C3-153, Dunav, Balkan etc.

Resistance to ascochyta blight was associated with improved yields and seed quality and represented ecologically pure production.

Plant height and the degree of lodging were used to determine adaptability to mechanized production and harvesting. Plant height of the evaluated materials ranged from 55 cm (Balkan) to 75 cm (Progres) (Table 3). Varieties with pods situated high on the plant which is expected to prevent harvesting losses, were preferred in

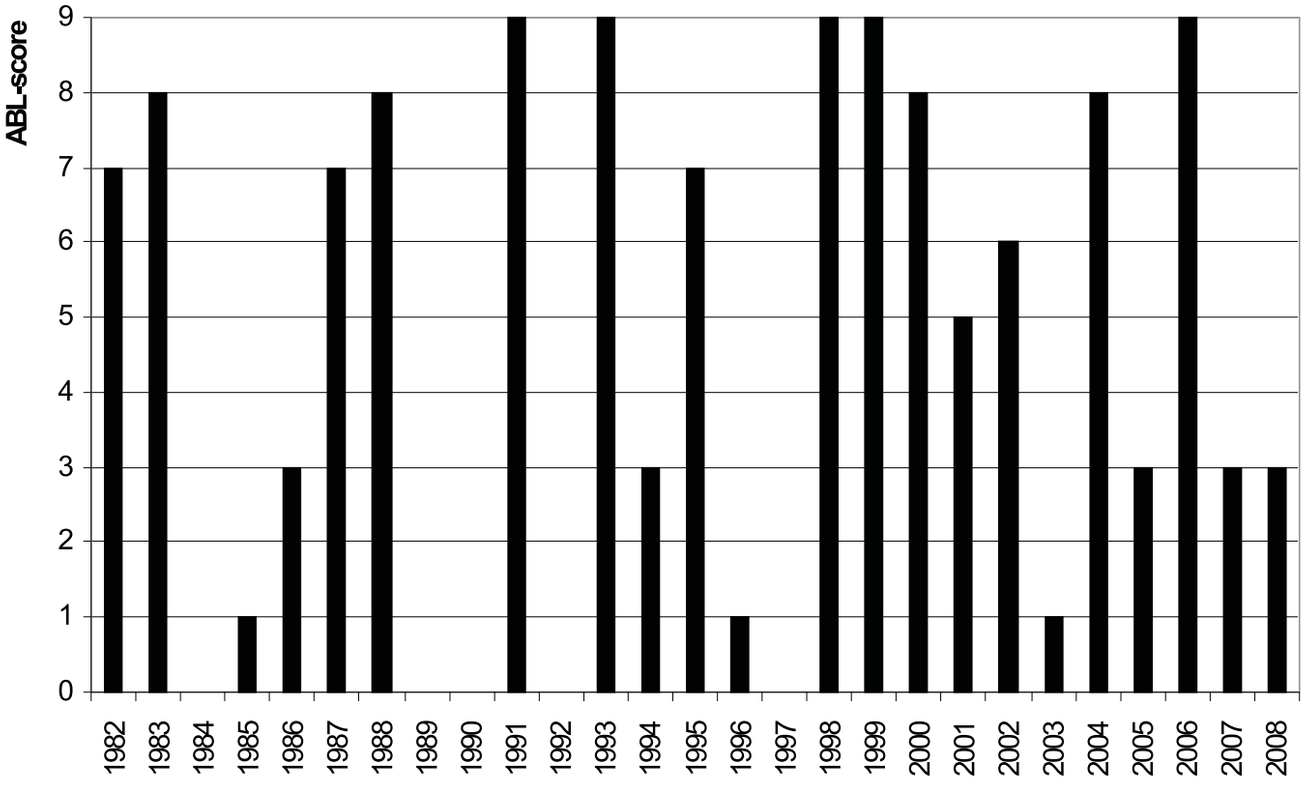

Figure 1. Disease reaction of the susceptible varieties
Resursi 1 and ILC 262 at Dobrudzha Agricultural Institute from 1982 to 2008.

practice. In rainy years, a big part of the tested materials lodged in different degrees, and were hard to harvest due to weed infestations.

It is very important that new varieties are tall, remain erect and so not lodge. From the materials tested, FLIP 95-58-1-2-1, Balkan, Progres, Dunav, FLIP 93-177c/50Gy, FLIP 92-162c-1-1-1 had the least amount of lodging.

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The weight of 1000 seeds of the new materials had a narrow range. The standard variety Stepnoi 1 was distinguished by having the lowest seed weight (300 g) and with smooth pea-shaped seeds. The seed weight of the remaining varieties and lines ranged from 349 to 428 g.

Chickpea productivity is the main breeding objective. It is genetically and environmentally determined and influenced by the prevailing agricultural practices. When yields were compared, the Bulgarian and foreign varieties and lines exceeded the yields of the standard varieties. The highest yield and good morphological and physical qualities was shown by the new varieties Balkan – 2910 kg/ha, Progres – 2720 kg/ha and candidate-variety Dunav – 3200 kg/ha. The yield of the standard varieties was 2420 kg/ha for Stepnoi 1 and 930 kg/ha for Resursi 1. Some of the promising lines had higher productive potential as X97C-3-153 – 3280 kg/ha, FLIP 93-177c/50Gy – 3220 kg/da, FLIP 93-177c/200Gy – 3210 kg/da,

Conclusion

For the period from 1982 to 2008 at Dobrudzha Agricultural Institute, General Toshevo a rich amount of over 14,200 lines of initial and breeding material was collected and created from local and foreign varieties, hybrid and mutant populations, and material received through expeditions in the country and through international collaboration mainly with ICARDA, Aleppo, Syria; USDA-ARS, Pullman, USA; Vavilov Institute – Russia and other sources.

Conditions were favorable for blight development in Bulgaria in 52% of the years from 1982 to 2008. Low infections were noted in 30% of the years and there was no disease in 18% of the years. In the latter years, conditions were not present for blight development.

Table 2. Field reaction to ascochyta rabiei of the new varieties and promising chickpea lines

Variety, line	ABL-score (0-9)			Average	Range
	2004	2006	2008		
Stepnoi 1 – St_1	0	7	1-3	2.13	0-7
Resursi 1 – St_2	9	9	3	4.50	0-9
Balkan	0	5	0-1	1.30	0-5
Progres	0 1	5	1 3	2.25	0 5
Dunav*	1-3	4	0	1.25	0-4
X97C-3-153	1-3	3	1-3	1.60	0-3
X97-C-25131	3	4	1-3	3.00	0-4
M99CC-17-1-2	0	6	1	1.13	0-6
M01CF-11-1-1-1	0-1	4	1	1.60	0-4
M01CF-14-3-1	0-1	4	0-1	2.00	0-4
FLIP 92-155c-1-1-7	0-1	3	0,3	0.86	0-3
FLIP 92-162c-1-1-1	0-1	5	0-1	1.50	0-4
FLIP 93 182c 3 2	0	1 3	1	0.63	0 3
FLIP 95-44c-32231	1-3	1-3	1	0.63	0-3
FLIP 95-58c-1-2-4	1-3	3	0	1.13	0-4

*candidate-variety for third year in test variety system

Table 3. Yield and other characters of new chickpea varieties and lines with resistance to ascochyta blight at Dobrudzha Agricultural Institute from 2002 to 2008

Variety, line	Yield kg/ha	Weight of 1000 eeds, g	Plant height,cm	Lodging
Stepnoi 1 – St_1	2420	300	66	0-7
Resursi 1 – St_2	930	410	57	1-5
Balkan	2910	392	55	0 3
Progres	2720	410	75	0 3
Dunav	3200	396	58	0 3
X97C-3-153 Obr. Chiflik 1/Sanford	3280	423	60	0-3
X97C -3-25131 Obr. Chiflik 1/Sanford	3075	428	59	0 7
M99CC-27-12 Stepnoy 1 x 50Gy+EMS 0.05%	2610	349	64	0-8
M01CF-11-11-1 FLIP 93-177c/50Gy	3220	383	62	0-1
M01CF-14-3 FLIP 93 177c/200Gy	3210	394	61	0-5
FLIP 92-155c-1-1-7	3120	400	57	0-9
FLIP 92 162c 1 1 1	3010	395	64	0 3
FLIP 95-44c-32231	3080	398	62	0-8
FLIP 95 58 1 2 1	3060	396	59	0 1
FLIP 93-182c3-2	3130	395	59	0-6

This analysis showed that Ascochyta blight is a major factor limiting chickpea production in Bulgaria and making resistance an important breeding objective.

The basic breeding methods in Bulgaria for creating genetic diversity were intervarietal hybridization and experimental mutagenesis.

Material collected within Bulgaria and obtained from outside sources, the breeding methods used, the international collaboration resulted in the development and registration of two new varieties – Balkan and Progres. Candidate-variety Dunav is for the third year in variety testing. All varieties have high productivity, from good to very good blight resistance, good seed quality traits, suitable for mechanized harvesting, and with good adaptability. Many lines with very good blight resistance were tested in the breeding nursery.

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