Identification Of Resistance To Downy Mildew In Sunflower

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Abstract

The method of lab-field suggested by Kukin (1966) was adopted to identify the downymildew disease resistance using 518 sunflower accessions in the present study. The disease-resistance variety RHA 274 and the diseased variety 'Peredovik' were used as check samples respectively. The sunflower seedlings (with radicles 3 to 10 mm) were inoculated with zoosporangial suspension. Investigate the percentage of diseased plant. The result revealed that 55 lines have disease resistance to Race 1 and Rce 2, 11 lines may have resistance genes, the others were diseased susceptible varieties. Investigations of the hereditary basis in the disease-resistance variety shows that in the procedure of breedings ideal results can obtain by using the resistance lines of wild species or clecting wild species of the sunflower genus.

Key Words: Downy mildew, sunflower diseases, downy mildew races

Sunflower (*Helianthus annuus* L.) is a new oil plant developing since 70's. In 1985 its plant area was more than that of sesame (*Sesamum indicum* L.) (a traditional oil plant in China) and so it became the 3rd. important oil plant in China. Because of the merits of drought-enduring, salt-enduring, poor-soil-enduring and high-oil content, its plant area became very large in the North of China. At the same time, the disease in sunflower had a trend of increasing year by year. Nowadays sclerotinia wilt, downy mildew and alternaria blight in sunflower had become the main diseases and caused large loses of crops in

the year suitable for the epidemics of disease. So 'The selective breeding of new variety with the character of deisease resistance in sunflower' had become a key study of the Chinese Ministry of Agriculture during the 8th Five-Year Plan. Coorperations between several breeding units were established to gain new variety or hybrid strain with resistance to one or several major disease in sunflower.

Downy mildew, incited by *Plasmopara halstedii* (Farl.) Berl. & de Toni, is known to occur almost everywhere in the world where sunflower is grown. In Canada, the diseased plant had once increased from several to almost 95%of the total plants. In the sunflower field of Red River Valley in U. S. , the diseased plant had ever been to 90%[1] of the total. In East Europe, the disease was discovered first in 1941, while in many field of Yugoslavia (1952), Bulgaria (1962), former Soviet Union (1965), the diseased plant reached 90% and caused a drop in production by 50%～90%. In China the disease was found first in Heilongjiang Province in 60's. Since then, the disease spread to Jilin (1972), Xinjiang (1974), Liaoning (1981), Shanxi (1986). In 1983, several fields were ruined completely in Baicheng, Qianguo, Fuyu, Nongan in Jilin Province[2]. In Shanxi Province, the disease was first discovered in Xinzhou city and spread to six counties. It harmed 6, 700 ha sunflower and the diseased plant reached 4～15%commmonly or 43～79% in the serious infected fields[3]. Seeing that, selecting the new variety with resistance to downy mildew became one of the main work of the coorperative groups.

Antigen with gene of resistance to downy mildew was necessary in the selecting. In Liaoning Province there are 280 accessions of sunflower germplasms, which are brought into the State Permanently Preserve Plan (SPPP), and several thousands of original materials of sunflower in Liaoning Academy of Agricultural Sciences. These materials need to be identified. The objects of this study were to identify the characters of resistance to downy mildew in the sunflower germplasms now available using the method of artificial infection in order to screen germplasms with resistance for the use of selecting new variety.

Materials and Methods

In the second ten days of June, 1995, when there were serious downy mildew in the continuous cropping sunflower identification garden, the method of lab-field suggested by Kukin (1966) was adopted to identify the disease resistance using 240 accessions of sunflower germplasms (brought into SPPP) and 278 accessions of original materials. The disease- resistance variety RHA274 and the diseased variety 'Peredovik' were used as check samples respectively. 100 seeds of each variety were rolled up in ragdoll, then immersed in water for 12 h at room temperature (22～26℃). After that twisting the ragdoll slightly to exclude the surplus water and germinating at room temperature. About 24 h later, the seeds began to sprout. Germinated seeds with radicles ≈ 3 to 10 mm in length were peeled and put into a nylon string bag according to the variety, immersing in prepared zoosporangial suspension for 24 h to inoculate at 18～22℃. The preparing method for zoosporangial suspension was as follow: After immersed the seeds for 12 h and germinated for 12 h, diseased plants were taken from the field and put into a plastic tub with some water in it. The plants were incubated with moisture-tight for 12h so that zoosporangial can grow in the diseased leaves. Then wash out the zoosporangial with distilled water and exam the zoosporangial under microscope. Try to let the suspension containing 10,000 zoosporangial mL^{-1}. Rest quietly for 1 h so that zoospores can grow. Thus the suspension can be used to inoculate the seedlings.

The inoculated seedlings were planted in the field. Random arrangement without replications. One row for each variety with a lenghth of 50 cm and a sowing width of 10 cm. Keep the soil wet by spraying water on time. One week after emergence, the seedlings were watered and covered with plastic film for 24 h. Then a white layer of mould composed of conidiophore and conidium was grown on the cotyledons of the infected seedlings. This kind of plants were ascertained as diseased plant. Investigate the percentage of diseased plant of each variety. If the percentage was zero, the variety belonged to 'R' (disease resistant). If not, 'S' (disease susceptible). But if it lowered than

30%, we listed this variety 啊 'S+' (maybe they had resistance genes).

Results and Discussion

The percentage of diseased plant in RHA274 control variety of resistance is zero, while that in 'Peredovik' control variety of susceptible is 100%. Among the 240 accessions of sunflower germplasms in Liaoning Province (belonging to SPPP), 155 are native varieties and improved varieties, the other 85 are introduced varieties from abroad. Among this 155, only 4 improved lines are 'R', and 15 out of the 85 introduce varieties are 'R' (Table 1). 37 lines out of the 278 original materials are 'R' (Table 2). 11 out of the 240 pieces of germplasms are 'S+'. The others are deseased varieties (Table 1).

Investigation of the hereditary basis in the 4 'R' out of the improved varieties shows that 293—8S comes from Jerusalem artichoke (*Hilianthus tuberosus* L.) ×8241, H14-2-5S comes from wild-breed ×7804-3, H21-1-5S derived from wild-breed. These three 'R' lines have the genetic genes of the wild spedies. The parents of another 'R' line, 7804—1, are 'Milasul' and Baikui No. 3, the former is a 'R' line introduced from France. The investigations of 37 'R' lines (table 2) out of 278 original materials shows that their parents are either Jerusalem artichoke, *H. argophyllus* Torrey & Gray or 'R' lines which the basis are not clear. Only 92228—2—2—1 is selected from a hybrid ST314 introduced from U. S.. The hereditary basis of ST314 is not know. As it well known, all the *Pl* genes identified so far come from wild species of *Helianthus*[4]. Our results confirm this also. So we suggest in the procedure of resistant breedings ideal results can obtain quickly by using the 'R' lines of wild species or electing wild species of sunflower genus.

Resistance to downy mildew was controlled by a single dominant gene *Pl* (Vranceanu et. al., 1970). Befor 1980, two races of sunflower downy mildew were identified. Six known races were listed in 1990 (W. E. Sackston et. al.)[5] and nine races in 1992 (T. Gulya) (personal communication). Identification of the race of sunflower downy mildew in the disease-resistance identified garden in Liaoning Academy of Agricultural

Sciences shows that the race is Race 2 (Red River Race) (Weishouen, et. al. 1994[6]). In the present study, the pathogenetic fungi for both inoculation and race identification came from the same sector in the same field. So the 'R' variety identified is resistant to Race 2 exactly. According to the research of predecessors, this variety is also resistant to Race 1. Further research is needed to identify whether it is resistant to other known races or not.

According to the study of Vear (1978), spores will grow on the cotyledons 2 weeks after inoculation in some sunflower varieties. 4 weeks later the cotyledons died while the plant grew normally. There were differences on the degree of resistance between the lines with the same *Pl* genes. Since the main purposes of the present study are to select the 'R' lines, studies about these problems do not accomplish. Further more, our test conditions are poor (compared with the best standard listed in the literatures, e. g. the temperatures during the inoculations are higher than the best ones (15～18℃) because of lacking of equipments. So the percentage of diseased plant in 'S' varieties do not reach 100% just like that of the control 'Peledovik'. In any case, the 'R' varieties identified in the present study with zero percent of diseased plant must be resistant and can be used in the breeding procedure of disease-resestance varieties. Those materials with less than 30% of diseased plant may have disease-resistance genes in heterozygosis state. The disease-resistance lines are gain through purifying the resistant genes. The methods are as follow. Sowing seed in the field after artificial infection. Selfing with resistant plant. Infecting again using the selfed seed. After several generations the disease-resistance lines are gained. The percentage of diseased plant in some susceptible materials may not be 100% because of the poor test conditions.

Table1 Resistance to downy mildew in sunflower germplasms of SPPP

Number	Name of variety	Resistance
Z0436	Changtu xiangrikui No. 1	S
Z0437	Changtu xiangrikui No. 2	S
Z0439	Kaiyan xiangrikui No. 1	S
Z0440	Faku xiangrikui	S
Z0441	Kaiyan xiangrikui No. 2	S
Z0442	Kaiyan xiangrikui No. 3	S
Z0443	Kaiyan xiangrikui No. 4	S
Z0444	Yangguazi	S
Z0445	Kaiyan xiangrikui No. 5	S
Z0446	xiangrikui	S
Z0447	Maoke	S
Z0448	Laotouke	S
Z0449	Kuihuazi	S
Z0450	Wutoubai	S
Z0451	Baikui	S
Z0452	Maozike	S
Z0454	Xingcheng xiangrikui No. 2	S
Z0455	Jinxi xiangrikui	S
Z0456	Jinxian xiangrikui	S
Z0457	Jianchang xiangrikui	S
Z0458	Dazizhuanrilian	S
Z0459	Beipiao xiangrikui	S
Z0460	Dazi	S
Z0461	Zhuanrilianhua	S
Z0462	Zhuanxinlian	S
Z0463	Maozike	S
Z0464	Fushun xiangrikui	S
Z0465	Zhuanlian	S
Z0466	Gaiping xiangrikui	S
Z0468	Yinkou xiangrikui	S
Z0470	Xiuyan xiangrikui No. 2	S
Z0471	Xiuyan xiangrikui No. 3	S
Z0472	Zhuanghe xiangrikui	S
Z0473	Xiaolizi	S
Z0474	Zhuanrilian	S
Z0475	Huasexiangrikui	S
Z0476	Hualianxiangrikui	S
Z0477	Lushun xiangrikui	S
Z0479	Qingyuan xiangrikui	S
Z0480	Qingyuan xiangrikui	S
Z0481	Xifeng xiangrikui No. 1	S
Z0482	Xifeng xiangrikui No. 2	S
Z0483	Zhuanrilian	S
Z0485	Dalixiangrikui	S
Z0486	Native xiangrikui	S
Z0487	Andong xiangrikui	S
Z0488	Maozike	S
Z0489	Zhuanxilian	S
Z0490	Zhuanxilian	S
Z0491	Huihuaxiangrikui	S
Z0492	Kazuo xiangrikui	S
Z0493	Xiangyangzhuan	S
Z0494	Taian xiangrikui	S
Z0495	Kaiyuan xiangrikui	S
Z0496	Fuxin xiangrikui No. 1	S
Z0497	Fuxin xiangrikui No. 2	S
Z0496	Fuxin xiangrikui No. 3	S
Z0501	Fuxin xiangrikui No. 6	S
Z0502	Fuxin xiangrikui No. 7	S
Z0503	Fuxin xiangrikui No. 8	S
Z0505	Jiuliancheng	S
Z0506	Damaoke	S
Z0507	Baizi	S
Z0508	Jian	S
Z0509	Qihua	S
Z0510	Liaokui No. 1	S
Z0511	7601—5 S	S
Z0512	7601—6—2 S	S
Z0513	81—37 S	S
Z0514	81—31 S	S
Z0515	59—1 S	S
Z0516	60—2 S	S
Z0517	254—5 S	S
Z0518	328—2 S	S

Number	Name of variety	Resistance
Z0519	348—1 S	S
Z0524	279—1 S	S
Z0525	293—8 S	R
Z0526	47—2 S	S
Z0527	47—7 S	S
Z0528	322—2 S	S
Z0529	59—4 S	S
Z0531	59—3 S	S
Z0532	348—8 S	S
Z0533	349—6 S	S
Z0534	350—1 S	S
Z0535	64—1 S	S
Z0536	147—4 S	S
Z0537	372—10 S	S
Z0538	378—10 S	S
Z0539	377—2 S	S
Z0540	417—10 S	S
Z0541	395—10 S	S
Z0542	405—2 S	S
Z0543	409—1 S	S
Z0544	412—10 S	S
Z0545	415—1 S	S
Z0546	47—1 S	S
Z0547	339—7 S	S
Z0548	411—10 S	S
Z0549	292—10 S	S+
Z0550	375—3 S	S
Z0552	808—2 S	S
Z0553	143—3 S	S
Z0554	52—3 S	S
Z0555	33—7 S	S
Z0556	154 S	S
Z0557	99 S	S
Z0558	147 S	S
Z0559	169 S	S
Z0560	8384 S	S
Z0561	787—1 S	S
Z0562	167 S	S

Number	Name of variety	Resistance
Z0563	RO70	S
Z0564	RO71	S
Z0566	Peredovik	S
Z0567	Record	S
Z0568	7601—2 S	S
Z0570	7601—4 S	S
Z0572	7712—3 S	S
Z0573	7712—5 S	S
Z0575	7804—1 S	S
Z0576	7808—3 S	S
Z0577	7819—3—1 S	S
Z0580	8208 S	S
Z0581	H14—2—5 S	S
Z0582	H21—1—5 S	S
Z0583	78—63—17 S	S
Z0584	8329 S	S
Z0588	7613A	S
Z0589	7613B	S
Z0590	7614A	S
Z0591	7614B	S
Z0592	7715A	S
Z0593	7715B	S
Z0594	7718A	S
Z0595	7718B	S
Z0596	7724A	S
Z0597	7724B	S
Z0598	242A	S
Z0599	242B	S
Z0600	76055A	S
Z0601	76055B	S
Z0602	181	S
Z0606	Taiyuan	S
Z0607	Weishan	S
Z0608	Yingnan	S
Z0609	Xinjianghei	S
Z0610	Xinjianghua	S
Z0611	Hu 18	S
Z0612	081517+	S

Number	Name of variety	Resistance
Z0613	Shaxuan 49	S
Z0614	Chayouqianqi	S
W0162	Charata	S
W0163	Impira	S
W0164	Mei 75—1	S
W0165	BEHT3	S
W0166	861	S
W0168	Ah1—5	S
W0169	5aF2	S
W0170	P386	S
W0171	C003—1	S
W0175	8501	S
W0176	8502	S
W0178	8504	S
W0179	8505	S
W0180	8506	S
W0181	8507	R
W0182	8508	R
W0183	8509	S
W0184	8510	S
W0185	8511	S
W0187	8513	R
W0188	8514	R
W0189	8515	S
W0191	8517	R
W0192	8518	S
W0194	8520	R
W0194	8521	S^+
W0196	8522	S
W0197	8523	S
W0198	8524[a]	S[a]
W0199	8525	S
W0200	8526	S
W0202	8528	S
W0203	8529	S
W0204	8530	S
W0205	8532	S

Number	Name of variety	Resistance
W0206	8533	S^+
W0208	852058	S^+
W0209	P_2	S
W0210	P_4	S
W0211	P_5	S
W0212	P_6	S
W0213	P_9	S
W0214	P_{12}	S
W0215	P_{13}	S
W0216	Peredaick	S
W0217	S317	S
W0218	2133—1	S
W0219	S338	S
W0200	Victoria	S^+
W0221	X33	S
W0222	R2033	S
W0223	HA336	R
W0224	HA337	S
W0225	2119—2	S
W0226	2033—5	S
W0227	2124—2	S
W0228	2133—2	S
W0229	2134—2	S
W0230	2139—3	S
W0231	RHA274	R
W0232	HA340	R
W0233	2225—1	S
W0234	2226—1	S
W0235	HA388	R
W0236	HA89A	S
W0237	HA89B	S
W0238	R018	S
W0239	R022	S
W0240	R029	S
W0241	R055	S
W0242	R056	S
W0243	HS301	S

Number	Name of variety	Resistance
W0244	Shangkui No. 1	S
W0245	Shangkui No. 2	S
W0246	Mexico	S
W0248	Ukraine	S
W0249	77—22	S
W0250	RHA271	R
W0251	DM2	R
W0253	HAR4	S^+
W0254	HAR5	S^+
W0258	CM29	R
W0259	953—88—31—54	R
W0261	8643—1	S

Table 2 Lines of resistance to downy mildew in native breeding materials of sunflower

name of line	source
85313	TUB* × PERBC$_4$/Milasul
92322	ARG* * × PERBC$_2$
92238	TUB × P“”BC$_3$/ARG × PER17602
92241-1	〃
92363-2	〃
92389-7	〃
92443-1	〃
9282—1-2	TUB × PERBC$_3$
92174—1-2	TUB × PERBC$_4$
92233-2—10—1	〃
92233-2—10—4	〃
92234-2—5	〃
92228-2—2—1	ST314 (a hybrid coming from America)
92232—1—4—2	TUB × PERBC$_4$/France
92232—1—4—4	TUB × PERBC$_4$/France
944155-2—1	〃
944156-2—9	〃
944157	〃
944158	〃
944159	〃
944160	〃
944161	〃
944163	〃
944164	〃
944167	〃
944168	〃
944170	〃
944171	〃

name of line	source
944197-2-7	"early" ×7602-15/RHA274
944195-2-7	TUB×PERBC$_3$/L68/RHA274
2623-1-2	7718B transformed into *H. tuberosus* DNA
2623—1—6	〃
2623—2—4	〃
231—1—6t	Vk4640087/8425R
251—1—1t	Vk45480017/8425R
8411	HA89A×(France×Baikui No. 3)
8425	109A×(7614×RHA274)

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