

Seed pathology of sunflower downy mildew

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Seed-borne and seed-transmission of sunflower downy mildew (*Plasmopara halstedii*) were further defined through differential staining in this study, which demonstrated that this fungus grows in both inner tissue of pericarp and spermoderm inducing seed infection in 86.7-100%. The infection caused by *P. halstedii* transmitted by seed showed symptomless (latent) although the incidence of seed-transmission is 44.4-91.3%. The pathogen also infects root and basal stem.

Key words: Sunflower, Downy mildew, Seed-borne, Seed-transmission, Latent infection

Sunflower downy mildew (*Plasmopara halstedii* (Farl.) Berl. & de Toni) is a systemic and destructive disease, it infects root, stem, leaf, flower and fruit. The pathogen can be spread over long distance by seed inducing its establishment and development in the new areas.

Sunflower downy mildew occurs sporadically in sunflower production areas in China, it is a new quarantine disease which needs to be controlled. Therefore, seed-borne and seed-transmission are the principal aspects for plant quarantine.

Materials and Methods

1. Materials

Infected seed of sunflower varieties: San-Daomei (from Yuanping of Shanxi Province); Mokui and Kui-1 (from Xinzhou of Shanxi Province); farm varieties (from Yanqing of Beijing and Inner Mongolia Autonomous Region); Peredovik (from Shawan of Xinjiang Uygur Autonomous Region); Huakui (from Anshun of Guizhou Province).

Uninfected seed of sunflower varieties: Liaokui-Za-2 (from Shenyang of Liaoning Province); San-Daomei (from Yuanping of Shanxi Province); G101(F1) (from United States).

2. Methods

2.1. Seed-borne

Pericarp and spermoderm, stained with blue cotton-safranin after treatment with lactophenol, were controlled under a microscope. The intercellular mycelia and haustoria were blue and host cells were bright pink.

2.2. Seed-transmission

2.2.1. Seedlings examination: Infected seeds were sown in clay pots in growth cabinet at 20°C. Seedlings were observed after 7 and 10 days respectively. The pathogen was detected from seedlings when it was determined that sporulation occurred on the surface. The root (principal or lateral) and stem (from basal to growing point) were examined by differential staining under a microscope.

2.2.2. Mature plants examination: Infected seeds were sown in the field, sporulation was surveyed during growing season. Plants were examined at mid- and late-season. The epidermis of principal and lateral roots, basal and middle and top stems, xylem and pith were immersed in 15% KOH for one week, and then controlled under a microscope.

Results

1. Seed-borne

Total 280 seeds(30 batches) were separately examined to determine the morphology and regions of the fungus within seed and the infection severity. The amount of the fungus was recorded as varying levels 0, 1, 2 and 3.

All the seeds collected from infected plants carried *P. halstedii*, which was at inner tissue of pericarp and spermoderm. The amount of the fungus within seed varies as different parts, more in inner tissue of pericarp than in spermoderm. The percentage of fungus borne in inner tissue of pericarp was 86.7~100%, average 95.1% ; and 75.0~100% in spermoderm, average 91.1%. The morphology of the fungus in seed were non-septal intercellular mycelia and haustoria. Sporangia and sporangiophore were presented in some seeds, oospores occurred rarely and only observed at inner tissue of pericarp of one seed in all experiments.

We examined health seeds of two varieties from China and oil seed G101(F1) from USA, while no mildew was observed.

Table 1. Examination of seed infection with *Plasmopara halstedii*

Varieties	Inspected seeds	Spermoderm		Inner tissue of pericarp	
		Incidence(%)	Index	Incidence(%)	Index
Farm	72	100	90.7	100	100
Farm	32	81.3	51.0	93.8	61.5
San-Daomei	28	75.0	29.8	87.5	35.4
Kui-1	20	100	46.7	100	75.0
Mokui	15	80.0	26.7	86.7	33.3
Farm	20	100	78.3	100	68.3
Farm	10	100	40.0	100	80.0
Peredovik	20	80.0	35.0	90.0	68.3
Huakui	20	89.5	49.1	95.0	66.9
Liaokui Za-2	10	0	0	0	0
San Daomei	5	0	0		
(Health)					
G101(F1)	18	0	0	0	0
Average of infected seeds		91.1	59.9	95.1	67.8

2. Seed-transmission

The plants grown from infected seeds planted inside and outside grew well, no sporangia produced on leaves, nor were other symptoms including stunt observed. Such plants might grow to 2 m high and also flower, but the disc is less than health one and seed are not full formed.

The downy mildew presented in any stages of plant. The incidence of mildew in mature plant was 57.1% with oospores, sporangia, intercellular

mycelia and haustoria in root and basal stem. The incidence of mildew in seedlings was 44.4–91.3% with intercellular mycelia, haustoria and sporangia in roots and hypocotyl. Downy mildew produced more in root than in stem; more in principal root than in lateral; more in the basal stem than in top (table 2 and table 3).

Experiments demonstrated that the latent infection with downy mildew is particularly significant in the seed-transmission of the disease.

Table 2. Examination of the seedlings grown from infected seeds

Varieties	Total plants	Plants with sporulation	Plants with mildew inside	Rate (%)	Regions	Morphology	Rate (%)
Farm	23	0	21	91.3	PR	IM & H	91.3
					LR	IM & H	85.7
					BS 0-1cm	IM & H	73.9
					BS 1-2cm	IM & H	43.5
					BS 2-3cm	IM & H	30.4
					BS 3-4cm	IM & H	26.1
					BS 4-5cm	IM & H	9.5
Peredovik	18	0	8	44.4	PR	IM, H & S	44.4
					LR	IM, H & S	38.9
					BS 0-1cm	IM, H & S	27.8
					BS 1-2cm	IM, H & S	22.2
					BS 2-3cm	IM & H	5.6

PR: Principle root; LR: Lateral root; BS: Basal stem; IM: Intercellular mycelia; H: Haustoria; S: Sporangia.

Table 3. Examination of the mature plants grown from infected seeds

Varieties	Total plants	Plants with sporulation	Plants with mildew inside	Rate (%)	Regines	Morphology
San Daomei	6	0	4	66.7	Xylem of PR	O
					Epidermis of PR	IM, H & O
					LR	O
Mokui	2	0	1	50.0	Epidermis of PR	S
					Epidermis of BS	S
					Pith of BS	O
Farm	6	0	3	50.0	Epidermis of PR	IM, H & O
					LR	IM, H & O
Total	14	0	8	57.1		

PR: Principle root; LR: Lateral root; BS: Basal stem; O: Oospora; IM: Intercellular mycelia; H: Haustoria; S: Sporangia.

Discussion

We observed that the plant infected systemically by *Plasmopara halstedii* may produce infected seeds, the mildew produced more in shrivelled seed than in fully formed seed.

The differential staining used in our experiments is a new technique for seed examination described by us. It can be used to examine sunflower seed.

infected by downy mildew, it is an accurate and reliable method that may apply to plant quarantine extensively.

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