SEED-BORNE FUNGI ASSOCIATED WITH CAULIFLOWER SEEDS AND THEIR ROLE IN SEED GERMINATION

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ABSTRACT

Seeds of five Cauliflower cultivars Faisalabad Agaiti-1, Faisal Agaiti-2, Snow Drift, Local-1 and Shehzadi, used in this investigation were obtained from Taxilla, Abbotabad, and Khushab. A total of 15 seed samples were tested using the standard blotter paper method to find the seed borne fungi and their role in seed germination. Ten fungal genera were identified among the fungi isolated from seeds. Alternaria (14.8%) was the most common genus. The other common genera in order of prevalence were Helminthosporium (12.8%), Aspergillus falvus (8.2%), Rhizopus (5.6%), Curvularia (4.8%), A. niger (4.6%), Cercospora (3.8%), Fusarium (3.4%) and Chaetomium (3.2%). The fungal incidence (3.2 to 22 %) and range of occurrence (0 to 23) among identified genera was found variable. Among five cultivars tested highest fungal incidence of 10.1% with minimum seed germination of 78% was observed on the seeds of cauliflower cv.Local-1. While minimum fungal incidence of 6.5% and with maximum seed germination of 86% was observed on the seeds of Cauliflower cv. Snow Drift. All other three cultivars had intermediate fungal incidence and level of seed germination. These findings suggest the use of chemical treated seed for sowing to enhance the seed germination to get optimum crop stand. It also suggests to adopt precautionary measures to avoid the seed contamination by field fungi during threshing.

Key words: Cauliflower, fungal incidence, seed germination, seed health.

INTRODUCTION

Cauliflower (Brassica oleracea var. botrytis; Family: Cruciferae) is an important vegetable crop and is grown on an area of 22896 hectares producing 234664 tons annually in Pakistan (GOP, 2008-09). Its yield is lower compared to developed countries including USA, Australia, and China (FAO, 2009) due to abiotic and biotic constraints. Abiotic stresses include drought, poor soil fertility and mineral toxicity, low price to growers, high price of pesticide and fertilizer inputs, poor transportation and storage systems and inadequate irrigation water. Whereas biotic stresses consists of insects, nematodes, fungal, bacterial and viral disease, weeds, rodents, birds and non-availability of high-quality seed (Anwar and Iqbal, 2011). Seeds play a vital role in the production of healthy crops. They are carriers of some important seed-borne diseases caused by biotic agents, which results in considerable losses in yields (Anwar et al., 2012). The association of various fungi with vegetable seeds has been reported all over the world (Anwar et al., 1994; Al Kassim 1996; Elarosi, 1993; Haikal, 2008; Ellis et al., 1980). We did not observed uniform stands of cauliflower plants even when apparently good and healthy seed was planted on the grower’s field; often a poor stand is obtained which might be due to prevention of germination by rotting seeds and killing seedlings. It has been documented that much of the vegetable seeds failed to germinate but rotted because it was attacked by various seed-borne fungi (Al Kassim 1996; Esuruoso et al. 1975; Karwasra and Singh 1982). Seed- borne pathogens can affect the seed quality by damaging external or internal seed tissues and cause the important seed diseases like seed rot, seed necrosis, and seedling damage through the local or systemic infection (Bateman and Kwasna 1999; Khanzada et al. 2002). The present investigation deals with the isolation and identification of the fungi in samples of cauliflower seed obtained from three major vegetable production localities namely Taxilla, Abbotabad, and Khushab . The abundance of fungi was also observed on the seeds of five commercially available cultivars. A study of the comparison of seed germination of five cultivars in relationship to seedling health was also made.
MATERIALS AND METHODS

All seed samples tested in this investigation were obtained after harvest from cauliflower production regions located at Taxilla, Abbotabad, and Khushab. Five different commercial grown cultivars, Faisalabad Agaiti-1, Faisal Agaiti-2, Snow Drift, Local-1 and Shehzadi were studied for seed-borne mycoflora contamination. The seeds were assessed for the association of seed-borne fungi by standard blotter paper method (ISTA1985). The agar plate method and standard blotter technique as modified by Al Kassim (1996) were used (Muskett and Malone, 1941; de Tempe 1953). After one-week of incubation period, seed were examined under stereoscopic binocular for the presence of associated fungi. The isolated fungi were identified with the help of the keys (Raper and Fennell, 1965; Booth, 1971; Ellis et al., 1980; Barnett and Hunter, 1972; Sivaesan, 1990). A working sample of 400 seeds was taken at random from the samples of each cultivar. The seeds were surface sterilized by placing the seeds in clean strainers and immersing for 1 minute in a solution of 70% ethyl alcohol and then for 2-3 minutes in 1% sodium hypochlorite solution (1:4). Following sterilization the seeds were plated individually in sterilized Petri dishes using sterile forceps. Ten seeds were placed in each plate containing three layered water soaked blotter paper. The plated seeds were incubated at 25 ± 2 °C for one day. On the next day, seed were subjected to freezing to avoid germination. Seeds were allowed to incubate at 25 ± 2 °C for seven days following freezing. The seeds were examined under stereobinocular microscope for associated mycoflora. These fungi were cultured on potato dextrose agar medium (PDA) to sporulate. The species identification was made by examining under the compound microscope with the help of identification keys. The plated seeds were classified as infected and non-infected to determine the fungal infection incidence (Infected seeds /total seed) x 100).

Germination Bioassay: Germination test was carried out by using the standard rolled paper towel method (ISTA, 1985). Hundred seeds of each cultivar were randomly selected and were allowed to germinate between two blotter paper layers at 25 ±2 °C for seven days. At the end of incubation, the number of ungerminated seeds (including rotted seed) was counted using the ISTA rules 1993. The emerged seedlings were graded as normal (seedlings with well-developed root and shoot and free of disease symptoms) and abnormal (seedlings with under developed root, shoot or both exhibiting disease symptoms) defined by Anwar et al (1994). Fungi were examined under the stereobinocular on abnormal seedlings and rotten seeds. The diseased portions of the seedling including root and shoot were cut and plated on PDA medium to confirm the association of fungi. The different types of fungi obtained were subcultured on PDA and identified as above.

RESULTS AND DISCUSSION

Survey of Seed-borne fungi. The presence of fungi was observed from 15-seed samples of cauliflower collected from three production regions including Taxilla, Abbotabad, and Khushab. These samples were mixture of unknown cauliflower cultivars. When plated on moist blotter, ten fungal isolates belonging to seven genera were found to be associated with seeds included Alternaria, Cercospora, Fusarium Helminthosporium, Chaetomium, Aspergillus and Rhizopus. Four isolates including Alternaria, Cercospora, Fusarium and Helminthosporium were designed as field fungi, whereas other three Aspergillus, Chaetomium and Rhizopus were the storage fungi (Table 1). The highest percentage of infected seeds by Alternaria was 13-15% while fungal infection and incidence were 11-15 and 14.8%, respectively About 73-100 % of samples carried infection with these fungi. Tohyama and Tsuda (1995) found the association of A. brassicicola with 86% of the commercial Brassica cultivars (Table 1). Fifty percent of the seed samples showed the 3.4% incidence of Fusarium sp. with a range of 00-08. Curvularia sp. was recorded on five seed samples with a range and incidence of 00-07 and 4.8%, respectively. Elizabeth et al. (2008) reported that Alternaria, Curvularia and Fusarium associated with seed caused the deterioration of the seed quality. All the fifteen seed samples showed the recovery of Helminthosporium sp. with 12.8% incidence and with a range of 11-15. Valkonen and koponen (1990) reported that Helminthosporium sp. was responsible for 30% and 17% for pre-emergence injuries and post-emergence mortality of cauliflower seedling, respectively. Six seed samples yielded Cercospora spp. with an incidence of 3.8% and range of 00-06. Chaetomium sp. was found on four seed samples with a range of 00-07 and an incidence of 3.2%. Two species of Aspergillus including A. flavus and A. niger on almost all seed samples with an incidence of 8.2% and 4.6% and a range of 05-12 and 00-12, respectively. Jain and Pathak (1996) reported that A. flavus produces the toxic metabolites that results in the reduced shoot and root elongation while Ijaz et al. (2001) reported that A. niger is a damaging storage fungi that affects the seed quality and reduces seed germinability. Rhizopus sp. another storage fungus was founding on thirteen seed samples of cauliflower, which had an incidence of 5.6% and a range of 03-07. Badau (2006) reported that Rhizopus sp causes the
food spoilage and render it fatal to human beings. Seeds infect by R. stolonifer caused sunflower head rot leading to reduced sunflower seed yield and quality (Yildirim et al., 2010). Aspergillus niger, A. flavus, and R. stolnifer has been found associated with lentil seeds (Hussain et al., 2007; Vishunavat and Shukla, 1983). Five commercially grown cultivars including Faisalabad Agaiti-1, Faisal Agaiti-2, Snow Drift, Local-1 were used in this investigation. The comparative; seed health, range of fungal infection, incidence and abundance of

| Table 1. Range of infection and incidence of fungi recovered from the naturally infected seeds of cauliflowers |
|-----------------|-----------------|-----------------|-----------------|
| Fungi Recovered | Infected Samples | Range of Infection | Incidence (%) |
| Alternaria alternata | 13 | 11-18 | 14.8 |
| Alternaria sp. | 15 | 19-23 | 21.0 |
| Fusarium sp. | 07 | 00-08 | 3.4 |
| Curvularia sp. | 05 | 00-07 | 4.8 |
| Aspergillus flavus | 15 | 05-12 | 8.2 |
| A. niger | 14 | 00-12 | 4.6 |
| Rhizopus stolonifer | 13 | 03-09 | 5.6 |
| Cercospora spp. | 06 | 00-06 | 3.8 |
| Helminthosporium sp. | 15 | 11-15 | 12.6 |
| Chaetomium sp. | 04 | 00-07 | 3.2 |

Table 2. Seed health, range and incidence of associated fungi with the seeds of five cultivars of cauliflower.

<table>
<thead>
<tr>
<th>Cultivars tested</th>
<th>Seed Health</th>
<th>Range of fungi</th>
<th>Incidence %</th>
<th>Associated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faisalabad Agaiti-1</td>
<td>73</td>
<td>01-22</td>
<td>9.0</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., Curvularia sp., Aspergillus niger, A. flavus, Rhizopus sp., Helminthosporium sp., Chaetomium sp.</td>
</tr>
<tr>
<td>Faisalabad Agaiti-2</td>
<td>76</td>
<td>02-22</td>
<td>7.4</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., Curvularia sp., Aspergillus niger, A. flavus, Rhizopus sp., Helminthosporium sp.</td>
</tr>
<tr>
<td>Snow Drift</td>
<td>55</td>
<td>00-19</td>
<td>6.5</td>
<td>Alternaria spp., Aspergillus flavus, Rhizopus sp., Cercospora spp., Helminthosporium sp.</td>
</tr>
<tr>
<td>Local-1</td>
<td>71</td>
<td>03-23</td>
<td>10.1</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., Curvularia sp., Aspergillus niger, A. flavus, Rhizopus sp., Cercospora spp., Helminthosporium sp., Chaetomium sp.</td>
</tr>
<tr>
<td>Shehzadi</td>
<td>78</td>
<td>01-20</td>
<td>8.3</td>
<td>Alternaria sp., Fusarium sp., Aspergillus niger, Aspergillus flavus, Cercospora spp., Helminthosporium sp., Rhizopus sp.</td>
</tr>
</tbody>
</table>
Table 3. Germination of and health of emerged seedlings from naturally infected seeds, and association of fungi with abnormal seedlings of five cultivars of cauliflower.

<table>
<thead>
<tr>
<th>Cultivars tested</th>
<th>Germination %</th>
<th>Normal seedlings</th>
<th>Infected seedlings</th>
<th>Associated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faisalabad-Agaiti-1</td>
<td>85</td>
<td>74</td>
<td>11</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., Cercospora spp., Helminthosporium sp., Chaetomium sp.</td>
</tr>
<tr>
<td>Snow Drift</td>
<td>86</td>
<td>76</td>
<td>10</td>
<td>Alternaria sp., Fusarium sp. Cercospora spp., Helminthosporium sp.</td>
</tr>
<tr>
<td>Local-1</td>
<td>78</td>
<td>65</td>
<td>13</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., Curvularia sp., Helminthosporium sp., Chaetomium sp.</td>
</tr>
<tr>
<td>Shehzadi</td>
<td>80</td>
<td>68</td>
<td>12</td>
<td>Alternaria alternata, Alternaria spp., Fusarium sp., sp. Helminthosporium sp.</td>
</tr>
</tbody>
</table>

fungi among five cultivars is shown in Table 2. Data presented showed a differential inconsistency in respect of seed health, the range of fungal infection, and incidence but seed of all five cultivars were found almost infected by the same fungal isolates. Cauliflower cv. Faisalabad agaiti-1 showed 9% fungal incidence with a range 01-22. A 7.4% fungal incidence and a range of 02-22 were observed on Faisalabad Agaiti-2. The seeds of the cultivar Snow Drift showed the minimum fungal incidence of 6.5% with a range of 00-19 while maximum fungal incidence of 10.1% was found on Local-1 with a range of 03-23. The fungal incidence of 8.3% and a range of 01-20 were noticed on cultivar Shehzadi. Similar results have also been reported by other workers for seed borne fungi associated with Brassica (Humpherson-Jones 1980; Sivapalan and Browning, 1992). Pre-emergence losses in common bean were recorded when Fusarium infected seeds were sown (Hassan, 1999). The seeds of five cauliflower cultivars were evaluated to assess their germination potential, health of emerged seedlings from naturally infected seeds, and association of fungi with abnormal seedlings (Table 3). The maximum seed germination of 86% was noticed in Snow drift with a total of ten infected seedlings while minimum seed germination of 78% with a total of 13 infected seedlings was observed on the Local-1. Whereas 85% germination was found in Faisalabad Agaiti-1 with 11 infected seedlings while Faisalabad Agaiti-2 showed 84% germination with 10 infected seedlings. The seeds of the cultivar Shehzadi showed 80% seed germination with 12 infected seedlings. The rest of the seeds that did not germinated either had physical injuries or were rotted. The most common fungi associated with the infected seedlings were species of Alternaria, Curvularia, Fusarium, Chaetomium, and Helminthosporium. The infected seedlings showed the symptoms including dark stem lesions and damping-off seedlings coupled with stunted root and shoot growth. The association of the same fungal isolates with abnormal seedlings as that of seeds suggests that the seed borne fungi are involved in the development of abnormal seedlings. Seed health plays an important role in any crop production system. The goals of better yield cannot be achieved without the use of disease free seeds as they serve the important source of spread of various diseases. Porter (1939) and subsequently Leach (1960) emphasized the necessity to examine the samples of seed lots for the presence of saprophytic and pathogenic microorganisms before planting. This study showed the association of the two different types of fungi i.e. field fungi and storage fungi with the cauliflower seeds. The seed association of field fungi like Alternaria, Curvularia, Fusarium, and Helminthosporium is of great concern as they have a tremendous role in the poor seed germination and seedling diseases. Alternaria and Fusarium seed infection encouraged the Aflotoxin production, which had an impact on seed health and negative role in seed germination (Ozcelik et al., 1990). Alternaria has been reported to be a major cause of leaf spots in several field and vegetable crops (Puckdeedindan, 1966) and
the production of selective and non-selective toxins in vegetable and field crops (Bains and Tewari, 1987; Ontani et al., 1995; Ozcelik et al., 1990). These toxins induce injure leading to mortalty of plants (Robeson and Strobe, 1985). The species of Fusarium and Helminthosporium are potential plant pathogens, which are involved in inducing seed rot, seedling blight, pre and post emergence injuries to the seedlings in cauliflower and wilt on many field and vegetable crops (Begum et al., 2008; Karim, 2005). Sivanesan (1990) reported that the Curvularia sp. associated with seed, the cause the leaf spots, leads to abnormal seedlings emergence. Storage fungi viz., Aspergillus, Chaetomium, Nigrospora, Penicillium and Rhizopus have been reported to have negative effect on the viability of seed (Malaker et al., 2008). We isolated three fungal isolates from cauliflower seeds including Chaetomium, Aspergillus, and Rhizopus. Rhizopus stolonifer, the cause of sunflower head rot, induces seed hardness and reduces the seed viability; the use of such seed reduced sunflower seed yield and quality (Malaker et al., 2008). The infection by storage fungi not only responsible for qualitative and quantitative losses but also induce the production of mycotoxin responsible for economical injuries and public health risks for animal and human (Sacchi et al., 2009). These findings of this investigation provide important information on field and storage fungal pathogen of cauliflower seed, which are limiting factor in yield. The field fungi cause diseases like leaf spot, seedling blight and pre and post emergence of seedlings leading to poor crop stand and subsequently yield is reduced (Bolkan et al., 1976; Elarosi, 1993; Ellis et al., 1980; Hassan, 1999). Whereas the storage fungi deteriorate the seed viability and quality, which reduce crop stand and also render the seed unfit for human and animal consumption (Ontani et al, 1995; Ozcelik et al., 1990). This study also suggests strongly that seeds should be stored under recommend storage conditions. Further the grower must use fungicide treated seed to avoid seed infection from seed as well as soil borne pathogens to get maximum seed germination, healthy seedlings, improved crop stand and ultimately enhanced harvested yield.

REFERENCES


