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CO-RELATION BETWEEN TEXTURAL MORPHOLOGY OF SEEDS AND SEED MYCOFLORA OF PULSES

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ABSTRACT
Seeds of pulses are naturally infected during cultivation or transient storage periods. The infections involves metabolic interactions between fungal pathogen and seed cells. The metabolic interaction milieu generates various metabolites. These metabolites produces morphological expressions on the seed. There is a correlation between seed texture, coloration and seed mycoflora.

KEYWORDS: Seed mycoflora, seed texture.

INTRODUCTION

Pulses under study are Green gram (*Vigna radiata* L.), Black gram (*Vigna mungo* L.), Chickpea (*Cicer arietinum* L.) and Pigeon pea (*Cajanus cajan* L.) constitute an important crop in Maharashtra because of its protein, carbohydrate, vitamin and mineral content.

Green gram (*Vigna radiata* L.)

Green gram (*Vigna radiata* L.) is an annual plant with herbaceous bushy appearance. It attains a height of 1-3 feet, being more or less erect. The axillary raceme inflorescence is with variously yellow colored flowers in cluster. The fruit is typically a slender pod, measuring 3-4 inches long and bearing small, slightly flattened, globular seeds. The seeds are usually green in color but the cotyledons are used as *dal*. The plant requires 25-35 inches rainfall. It is cultivated both as a Kharif as well as Rabi crop. The Kharif crop is sown around June or July and Rabi crop in September or October. Within three months, the plant is harvested. Seeds show 24 g protein, 56.7 g carbohydrate/100g of edible part of the seeds, thiamin (0.47mg), riboflavin (0.27mg) and iron (7.3mg). (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Black gram (*Vigna mungo* L.)

Black gram (*Vigna mungo* L.) is an herbaceous annual plant with spreading procumbent branches, commonly referred as 'wooly pyool' due to presence of brown hairs covering stem. Inflorescence is represented by a long stout, hairy axis bearing a group of 5-6 yellow flowers. In India it is commonly grown as a kharif crop where rainfall is 30-35 inches. Usually cultivated in June -July and harvested within 3-4 months. Commonly cultivated in Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, West Bengal, Andhra Pradesh and Karnataka. Black gram is important for its high phosphoric acid content. It contains 24g protein/100g of seeds and carbohydrates 59.6g/100 g of seeds show that it is nutritious pulse. It also has good amount of phosphorus (385mg) iron (10.2mg), thiamin (0.42 mg), riboflavin (0.20mg), niacin (2mg) and vitamin C (3mg) (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Chick pea (*Cicer arietinum* L.)

Chick pea (*Cicer arietinum* L.) is small much branched plant attaining height of about 2 feet. The leaves are pinnately compound with Papilionaceous solitory flowers and the pods contains one or two seeds. It is cultivated in dry cool climate during Rabi season in the regions with low to moderate rainfall as intercrop along with Jowar, Wheat, and Bajra etc during October-November. The crop is harvested after about 3-4 months in February – March. It is mainly cultivated in Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar, Maharashtra, Andhra Pradesh, West Bengal, Tamil Nadu and Karnataka. Malic and oxalic acids from the leaves of Chick pea are useful in intestinal disorders. It contains protein 20.5g/ 100g of seeds and carbohydrates 59.6 g/ 100g of seeds with thiamin (0.30mg), riboflavin (0.15mg), niacin (2.9mg), vitamin C (3mg) and phosphorous (312 mg) (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Pigeon pea (*Cajanus cajan* L.)

Pigeon pea (*Cajanus cajan* L.) is an annual shrub of about 6-7 feet. The inflorescence is a typical axillary raceme bearing Papilionaceous flowers. It is cultivated as a mixed crop with Kharif cereals in low rainfall areas. Sowing is done in June – July and harvested after 6-8 months, between January- February. It is commonly

cultivated in Uttar Pradesh, Orissa, Rajasthan, Maharashtra, Bihar and Tamil Nadu. It contains protein 20.4 g/100 g of seeds and carbohydrates are 60.4 g/100 g of seeds, suggesting that it is also good source of protein and carbohydrates, it also contain thiamin (0.45mg), niacin (2-9mg) and riboflavin (0.19mg). It has better quality of fiber (7g/ 100g of seeds). (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Seed mycoflora of different categories of seeds such as bold, shrivelled, discoloured and cracked seeds have been studied. Sawhney and Aulakh (1980) studied fungi associated with normal and abnormal seeds of peas and their pathogenic potential. Ahmed *et al* (1981) studied mycoflora of cracked seeds of wheat and found that different fungi like *Penicillium* spp. *Aspergillus* spp. were responsible for the malformations of the test seeds. Randhawa and Aulakh (1984) studied mycoflora of discoloured and shrivelled seeds of pearl millet and found that seed mycoflora was responsible for the defect. Khairnar (1987) reported that, among different categories of mouldy seeds of bajra, the seeds with mixed type of discoloration were due to *Fusarium* spp. Tegge and Hiremath (1990) studied seed-borne fungi from shattering and non-shattering types of Green gram (*Vigna radiata* L.) and found that fungi associated were *Alternaria alternata*, *Cladosporium fulvum*, *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma roseum*. Further it is observed that, these fungi reduced seed germination and seedling growth. Danai (1994) reported that, the seeds of cracked and discoloured categories of jowar var. CSH-1 yielded maximum number of *Aspergilli* and also observed that, the bold seeds also showed fungi like *Aspergillus flavus* and *Aspergillus glaucus.* Umatale (1995) Studied groundnut and sunflower seeds and found that, discoloured seeds showed maximum incidence of fungi compared to wrinkled ones of Safflower and Sesamum. Waghmare (1996) Studied jowar var. CSH-1 seeds and found that, discoloured seeds were having highest counts of *Fusarium roseum* and *Fusarium semitectum*. Bodke (2000) studied different categories of seeds of cereals like wheat, bajra, jowar and maize and found that, discoloured seeds showed maximum number of fungi.

MATERIALS AND METHODS

Collection of seed samples

The methods prescribed by Paul Neergaard (1977) have been adopted for the collection of seed samples. Seed samples of Green gram, Black gram, Chick pea and Pigeon pea were collected from field, market places from Nanded. A composite seed sample for each of the pulse crop was made by mixing the individual seed sample together, preserved in gunny bags at room temperature during the studies.

Detection of seed mycoflora

The seed-borne fungi of different pulses, different categories and stored seeds of pulses were detected by moist blotter (B) and agar (A) plate methods as recommended by ISTA (1966), De Tempe (1970), Neergaard (1977) and Agrawal (1981). The procedure of moist blotter (B) and agar (A) plate methods is described as below.

Moist blotter plate method

In moist blotter plate method; a pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized borosil glass Petri-plates of 10 cm diameter. Ten seeds were placed at equal distance aseptically on the moist blotter paper. The plates were incubated at room temperature for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants for further studies.

Agar plate method

In agar plate method; 25 ml of sterilized Potato Dextrose Agar (PDA) medium of pH 5.6 was poured in pre-sterilized borosil glass petri-plate of 10 cm diameter. The petri-plates were allowed to cool at room temperature; then ten seeds of test pulses were placed at equidistance under aseptic condition. The plates were incubated at room temperature for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA slants for further studies.

RESULTS AND DISCUSSION

The Green gram seeds of all categories showed presence of *Aspergillus niger* (69%), followed by *Aspergillus flavus* (67%), *Aspergillus fumigatus* (62%), *Alternaria tenuis* (55%), *Drechslera tetramera* (56%), *Aspergillus carbonarius* (40%) and *Fusarium oxysporum* (40%). The fungi like; *Cladosporium* spp. showed its presence on bold and shrivelled seeds, *Colletotrichum truncatum* showed its presence on bold seeds and very less on discoloured and shrivelled seeds. *Macrophomina phaseolina* was present on all categories in minimum percent, but was absent on shrivelled seeds on blotter. *Alternaria alternata* and *Aspergillus nidulans* were absent on agar plates of discoloured seeds.

Table 1: Incidence of seed mycoflora of Green gram (*Vigna radiata* L.) seeds of different categories by blotter (B) and agar (A) Plate methods (after ten days of incubation).

Sr. No	Seed mycoflora	Incidence of seed mycoflora (%)					
		Bold seeds		Shrivelled seeds		Discolored seeds	
		B	A	B	A	B	A
1	*Alternaria alternate*	10	15	15	20	10	00
2	*Alternaria tenuis*	15	25	45	55	28	32
3	*Aspergillus carbonarius*	12	30	40	38	40	15
4	*Aspergillus flavus*	50	66	50	67	30	65
5	*Aspergillus fumigatus*	28	58	60	62	30	60
6	*Aspergillus nidulans*	35	25	10	55	15	00
7	*Aspergillus niger*	28	58	65	69	60	36
8	*Chetomium globosum*	15	00	10	16	00	13
9	*Cladosporium spp.*	00	12	00	02	00	00
10	*Colletotrichum truncatum*	10	00	00	03	00	02
11	*Curvularia lunata*	12	11	30	22	22	20
12	*Drechslera tetramera*	22	12	20	45	56	33
13	*Fusarium moniliforme*	20	37	35	18	20	21
14	*Fusarium oxysporum*	12	25	40	35	22	21
15	*Macrophomina phaseolina*	12	06	00	10	12	17
16	*Penicillium spp.*	00	12	00	20	00	17
17	*Rhizopus stolonifer*	22	09	40	12	12	45

The results clearly indicate that, shrivelled seeds of Black gram showed maximum mycoflora with maximum percent incidence; followed by discoloured and bold seeds. Total seventeen fungi were recorded from all categories of seeds. The predominant fungi among all categories were *Aspergillus niger* (80%), *Aspergillus nidulans* (70%), *Aspergillus flavus* (67%), *Aspergillus fumigatus* (66%), *Fusarium oxysporum* (60%), *Drechslera tetramera* (58%), *and Fusarium moniliforme* (50%). Minimum percent incidence was shown by C*ladosporium* spp. (1%), *Colletotrichum truncatum* (1%) and *Chetomium globosum* (3%). The agar plate method supported maximum growth of fungi compared to blotter method. *Alternaria alternata*, *Aspergillus carbonarius*, *A. fumigatus*, *Chetomium globosum*, *Cladosporium spp.*, *Colletotrichum truncatum* were absent on bold seeds plated on agar. *Chetomium globosum*, C*ladosporium* spp. *Colletotrichum truncatum* were not shown their presence on shrivelled seeds plated on blotter. *Alternaria tenuis*, *Aspergillus carbonarius* were absent on discoloured seeds plated on agar.

Table 2: Incidence of seed mycoflora of Black gram (*Vigna mungo* L.) seeds of different categories by blotter (B) and agar (A) Plate methods (After ten days of incubation).

Sr. No	Seed mycoflora	Incidence of seed mycoflora (%)					
		Bold seeds		Shrivelled seeds		Discolored seeds	
		B	A	B	A	B	A
1	*Alternaria alternate*	10	00	20	12	18	15
2	*Alternaria tenuis*	20	12	35	45	20	00
3	*Aspergillus carbonarius*	05	00	17	22	08	00
4	*Aspergillus flavus*	25	60	66	67	50	40
5	*Aspergillus fumigatus*	10	00	50	66	50	07
6	*Aspergillus nidulans*	15	66	70	70	40	55
7	*Aspergillus niger*	30	70	56	80	20	69
8	*Chetomium globosum*	12	00	00	03	05	13
9	*Cladosporium spp.*	00	00	00	05	00	01
10	*Colletotrichum truncatum*	11	00	00	02	00	01
11	*Curvularia lunata*	12	22	30	27	20	11
12	*Drechslera tetramera*	22	30	25	58	30	27
13	*Fusarium moniliforme*	18	22	50	38	25	20
14	*Fusarium oxysporum*	11	37	60	50	30	13
15	*Macrophomina phaseolina*	10	12	30	20	20	18
16	*Penicillium spp.*	12	12	30	20	20	18
17	*Rhizopus stolonifer*	02	15	05	18	01	22

The results clearly show that, in all sixteen fungi were reported on all categories of seeds Chickpea. Incidence of seed mycoflora was maximum on discolored seeds but percent incidence was maximum on shriveled seeds. Among the fungi, the predominant were *Aspergillus flavus* (80%), *Aspergillus niger* (63%), *Aspergillus fumigatus* (50%), *Drechslera tetramera* (55%), *Aspergillus nidulans* (55%) and *Fusarium oxysporum* (50%). The less predominant seed-borne fungi were; *Colletotrichum truncatum*, *Cladosporium* spp., *Rhizopus stolonifer*, *Macrophomina phaseolina*, *Aspergillus carbonarius*, *Alternaria alternata* and *Alternaria tenuis*. Seed-borne fungi like, *Cladosporium* spp. was completely absent on discoloured seeds but showed very less presence on shrivelled seeds (3% agar, 10% bold seeds, agar). Similarly *Colletotrichum truncatum* was absent on shrivelled seeds with less presence on discoloured seeds (1%) and bold seeds (3%). *Rhizopus stolonifer* was absent on shrivelled seeds with less percent incidence (2%) on discoloured seeds. Agar plate showed over all more incidence of seed mycoflora.

Table 3: Incidence of seed mycoflora of Chick pea (*Cicer arietinum* L.) seeds of different categories by blotter (B) and agar (A) Plate methods (After ten days of incubation).

Sr.No	Seed mycoflora	Incidence of seed mycoflora (%)					
		Bold seeds		Shrivelled seeds		Discolored seeds	
		B	A	B	A	B	A
1	*Alternaria alternate*	10	10	25	09	10	12
2	*Alternaria tenuis*	21	10	48	45	36	25
3	*Aspergillus carbonarius*	10	00	00	20	55	21
4	*Aspergillus flavus*	56	60	80	70	55	77
5	*Aspergillus fumigatus*	40	30	50	30	40	40
6	*Aspergillus nidulans*	20	22	40	18	55	23
7	*Aspergillus niger*	34	58	50	38	35	63
8	*Cladosporium spp.*	00	10	00	03	00	00
9	*Colletotrichum truncatum*	00	03	00	00	00	01
10	*Curvularia lunata*	40	06	40	20	30	19
11	*Drechslera tetramera*	50	12	50	40	50	55
12	*Fusarium moniliforme*	30	20	30	10	50	30
13	*Fusarium oxysporum*	21	25	50	35	30	28
14	*Macrophomina phaseolina*	10	00	22	15	15	10
15	*Penicillium spp.*	12	00	30	06	15	10
16	*Rhizopus stolonifer*	00	12	00	00	02	12

It is clear from the results that, among all categories of seeds, discoloured seeds of Pigeon pea showed more incidence of seed mycoflora (seventeen); with highest percent incidence. The predominant seed-borne fungi were *Aspergillus flavus* (80%), *Drechslera tetramera* (70%), *Aspergillus nidulans* (66%), *Aspergillus niger* (63%) and *Curvularia lunata* (59%). Minimum seed mycoflora was reported by *Cladosporium* spp., *Chetomium globosum*, *Colletotrichum truncatum* and *Rhizopus stolonifer*. Fungi like; *Chetomium globosum*, *Colletotrichum truncatum* did not show their presence on bold seeds. *Rhizopus stolonifer* did not appear on shrivelled seeds. Rest of the fungi showed their presence on all categories in more or less quantity. Agar plate showed more fungal growth as compared to the blotter plate method.

Table 4: Incidence of seed mycoflora of Pigeon pea (*Cajanus cajan* L.) seeds of different categories by blotter (B) and agar (A) Plate methods. (After ten days of incubation).

Sr.No	Seed mycoflora	Incidence of seed mycoflora (%)					
		Bold seeds		Shrivelled seeds		Discolored seeds	
		B	A	B	A	B	A
1	*Alternaria alternata*	05	10	15	16	20	12
2	*Alternaria tenuis*	15	25	14	45	30	55
3	*Aspergillus carbonarius*	00	25	12	30	32	22
4	*Aspergillus flavus*	60	40	60	73	80	75
5	*Aspergillus fumigatus*	35	25	50	48	50	55
6	*Aspergillus nidulans*	30	40	42	55	48	66
7	*Aspergillus niger*	35	50	34	60	40	63
8	*Chetomium globosum*	00	00	00	02	12	08
9	*Cladosporium spp.*	01	00	02	06	07	06
10	*Colletotrichum truncatum*	00	00	00	02	08	23
11	*Curvularia lunata*	25	18	34	45	36	59
12	*Drechslera tetramera*	39	30	70	58	40	60
13	*Fusarium moniliforme*	35	20	35	25	46	38
14	*Fusarium oxysporum*	40	30	20	40	30	40
15	*Macrophomina phaseolina*	10	00	05	15	22	28
16	*Penicillium spp.*	30	00	10	15	22	29
17	*Rhizopus stolonifer*	00	05	00	00	16	12

It has been proved that morphological textural features of the seeds of pulses are indicators of the internally located fungal infections. It is nothing but the seed-borne fungal pathogenicity causing malformation in the seeds of pulses. Roopam parashar et.al. (2019) reported thirteen genera and twenty three species of seed-borne fungi from four pulses, namely, Chickpea, Mungbean, Pigeon pea and Lentil. The seed-borne mycoflora consisted of *Aspergillus carbonarius, A. niger, A. fumigatus, A. flavus, Curvularia lunata* etc. These fungi caused adverse effects on pulse seeds. Rameela et.al. (2018) reported discolouration in Mungbean seeds due to seed-borne fungi.

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