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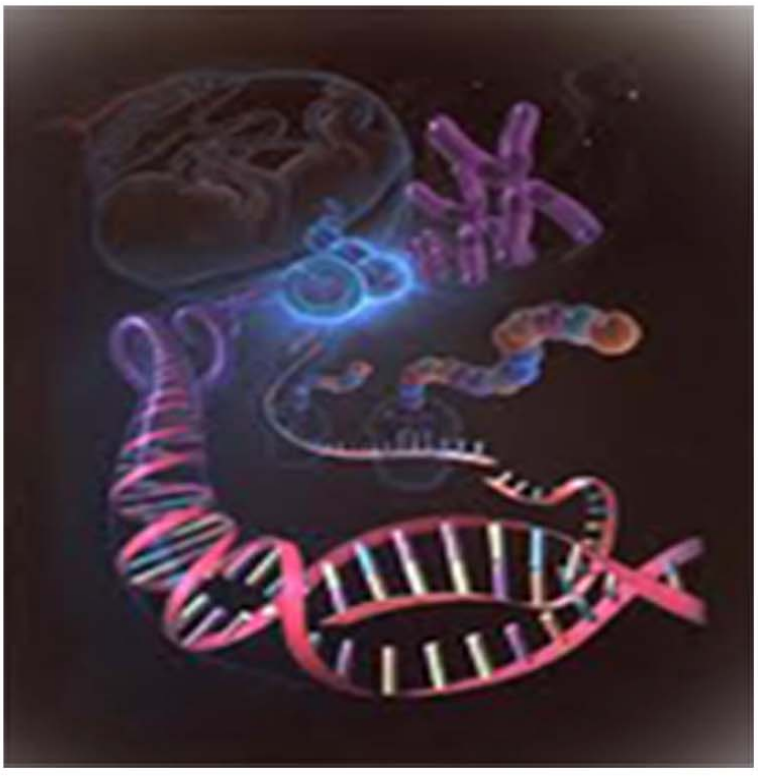

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Research Paper

ISOLATION, MORPHOLOGY, IDENTIFICATION OF PATHOGENS AND CONFIRMATION OF PATHOGENICITY OF DRY ROT, CHARCOAL ROT AND SOFT ROT PATHOGENS OF POTATO

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Potato (*Solanum tuberosum* L.) is one of the most nutritious sources of food in the world. Besides cereals, the potato is one of the crops, which can supplement food needs of a country. The dry rot, charcoal rot and soft rot pathogens were isolated and purified by tissue isolation technique. Pathogenicity test was confirmed through Koch's postulate's in respect of dry rot pathogen (*Fusarium* sp.) by tuber inoculation and soil inoculation technique, charcoal rot pathogen (*M. phaseolina*) by toothpick method and for soft rot pathogen (*E. carotovora*) slice method, injection method and pin prick method of inoculation were employed.

Keywords: Pathogens, Potato, Dry rot, Charcoal rot, Soft rot

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most nutritious sources of food in the world. Besides cereals, the potato is one of the crops, which can supplement food needs of a country. It has been recognized as a wholesome food and the richest source of energy in most of the countries of the world where, it forms an important part of the human diet. Potato easily stands out from this group of crops because of the distinct advantages it possesses as compared to other crops. This tuber which has sustained ancient civilizations and which continues to remain a major food crop throughout the world should no longer be considered a vegetable but instead be treated as a food crop. Potato tuber contains about 75 to 80% water, 16 to 20% carbohydrate, 2.5 to 3.2% crude protein, 1.2 to 2.2% true protein, 0.8 to 1.2% mineral matter, 0.1 to 0.2% crude fat, 0.6% crude fiber and some vitamins (Swaminathan and Pushkarnath, 1962). The quality of potato protein is also higher than that of most other food crops.

Potato crop has got immense potentiality for cultivation in Gujarat. The crop is mainly grown in *rabi* season, both under field as well as riverbed conditions. The state is very famous for its unique and model cultivation of potato under riverbed condition.

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Potato is prone to attack by more than hundred of diseases caused by fungi, bacteria, viruses, phytoplasma (mycoplasma) like microorganisms and nematodes that deteriorate quality and reduce yield of tubers. Unfortunately, many of them are tuber born in nature. Looking to the importance and available literature shows that no research work has been carried out on these diseases in North Gujarat condition and hence, it has been felt necessary to conduct systematic investigations on different aspects of isolation, identification, morphological and pathogenicity of potato tuber rot diseases.

MATERIALS AND METHODS

Collection of Potato Tuber Rot Infected Samples and Isolation of Pathogens

The naturally infected tubers showing the symptoms of dry rot, charcoal rot and soft rot diseases were collected from the Potato Research Station, Deesa. On confirming the symptoms as well as presence of fungal and bacterial pathogens on infected tubers, the samples were subjected to tissue isolation by cutting the infected tissue into small bits. The pieces were surface sterilized with 0.1% mercuric chloride solution for one minute followed by subsequent three washings of sterile distilled water and then aseptically transferred to sterile Petri plate containing 20 mL of Potato Dextrose Agar (PDA) for fungal pathogens and Nutrient Agar (NA) medium for bacterial pathogen. Inoculated Petri plates were incubated at 28 ± 2°C for the growth of the fungal and bacterial pathogens.

The respective pathogenic cultures were further purified and maintained on PDA and NA slants for fungal and bacterial pathogens. The periodical subculturing and multiplication were done on the same medium to keep the culture fresh.

Pathogenicity

Dry Rot

Pathogenicity test for dry rot under laboratory condition: The pathogenicity test of the dry rot pathogen (*Fusarium* sp.) was confirmed on ten freshly harvested healthy tubers of Kufri Badshah by inserting mycelium through minor injuries of 1-2 mm deep and the inoculated sites were sealed with wax. The inoculated tubers were incubated at 28 ± 2°C and were examined regularly for disease development. The pathogen was reisolated from diseased tubers after twelve days and compared with the original isolate of *Fusarium* sp. (Rai, 1982) while, ten potato tubers without inoculation were kept as control.

Pathogenicity test for dry rot under pot culture: In order to confirm pathogenic nature of *Fusarium* sp. in pot, the inoculum of *Fusarium* sp. was multiplied in Petri plates on PDA medium, which was further, used for inoculation. These inoculated Petri plates were incubated at 28 ± 2 °C temperature. The earthen pots of 45 cm diameter were sterilized by dipping them in 2% formaldehyde solution for about 1 min. The mixture containing soil, sand and farm yard manure in the ratio of 50:25:25 was filled in ten sterilized pots after autoclaving it for two times at 1.045 kg /cm^2 pressure for an hour and allowed to kept for 10 days to expel out harmful gases.

The mycelial suspension of *Fusarium* sp. was used for inoculation at the rate of 4 Petri plates/ pot. The inoculum of isolate was thoroughly mixed with the autoclaved soil mixture in eight pots and moistened with water before one week of planting for uniform spread of the inoculum. The remaining two pots were not inoculated with *Fusarium* sp. and kept as control for comparison. The potatoes of variety Kufri Badshah, weighing approximately

50 g, was surface sterilized with 2.5% sodium hypochlorite solution for five minutes before planting. Three potato seed tubers were planted in each pot. All the pots including check were irrigated regularly to maintain soil moisture. The pots were kept in green house for occurrence of the disease. The observations on the occurrence of the dry rot disease were taken at the time of harvesting of potato. At the end, reisolation was made from dry rot infected potato tubers harvested from inoculated pots for comparing the pathogen with the original isolate.

Charcoal Rot

In order to test the pathogenicity of *Macrophomina phaseolina*, healthy tubers of potato variety Kufri Badshah were used in the laboratory using the toothpick method of inoculation (Upadhaya, 1976).

Five potato tubers of variety Kufri Badshah were inoculated by inserting toothpick covered with mycelium of *M. phaseolina* 2 mm deep into the tubers. The inoculated tubers were incubated at 28 ± 2°C and examined after 10 days for disease development while, five potato tubers were inoculated by inserting only sterile toothpick which served as control. At the end, fungus was reisolated and compared with the original isolate of *M. phaseolina.*

Soft Rot

Slice method: Confirmatory test by inducing the soft rot pathogen (*Erwinia carotovora* subsp. *carotovora*) in healthy tuber slices was carried out by inoculating about 10^8 cells in the form of thick paste onto the surface of healthy tuber slices. The inoculated tuber slices were incubated for 24-48 h at 28 ± 2°C, in a two Petri plates having 5 mL of sterile water, kept in such a way that the tuber slices should not come in direct contact with the water. Tuber slices without inoculating the pathogen in one Petri plate was kept as control.

Softening of the inoculated tuber slices, accompained by production of characteristic odor was taken as positive reaction. From the experimentally induced bacterial pathogen, bacteria was reisolated and compared with the original isolate of *Erwinia carotovora* (Shashirekha *et al.*, 1987).

Injection method: The inoculum for soft rot pathogen was prepared by transferring one loopful of individual colony, growing on nutrient agar medium, to nutrient broth (10 mL), incubated at 25°C for 48 h and was used for inoculation. The nutrient broth was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and pellet again centrifuged twice at same rpm for 10 min with sterilized distilled water**.** The concentration of bacterial suspension was maintained 5 x 10^6 cfu/mL. This suspension was used as inoculum.

Six potato tubers of variety Kufri Badshah were washed with sterilized water and air dried before inoculation. Thereafter, bacterial suspension was taken in sterilized hypodermic injection syringe and needle was injected 20 mm deep in the tuber. Pressure on piston was maintained while, withdrawing the needle. The needle entry point was immediately sealed with wax. The inoculated tubers were incubated at 28 ± 2°C and examined after five days for disease development. Subsequently, four potato tubers were injected with sterilized Nutrient Broth only and kept as control. The pathogen was reisolated from rotten tissue of potato tuber and compared with the original isolate of *E. carotovora* subsp. *carotovora* (Kumar *et al.*, 1992).

Pin prick method: The bacterial suspension was prepared as mentioned in Injection method. Six potato tubers of variety Kufri Badshah were washed with sterilized water and air dried before inoculation. Here, a sterilized needle dipped in bacterial suspension was pricked 20 mm deep into the tissue. The pricking point was sealed with wax. The inoculated tubers were incubated at 28 ± 2°C and examined after five days for disease development while, four potato tubers were pricked with Nutrient Broth only which, served as control. The pathogen was reisolated from rotten tissue of potato tuber and compared with the original isolate of *E. carotovora* sub sp. *carotovora* (Kumar *et al.*, 1992).

Morphology and Identification of the Pathogens

Morphological characters of *Fusarium* sp. and *Macrophomina phaseolina* were studied by observing slides, stained with cotton blue under a microscope. Photomicrographs of mycelium and conidia were taken while, for bacterial pathogen *Erwinia carotovora* were studied by preparing a smear of bacterial suspension of *E. carotovora* on the slide. The slide was then examined under oil immersion.

RESULTS AND DISCUSSION

Collection of Potato Tuber Rot Infected Samples and Isolation of Pathogens

The tubers showing the infection of dry rot, charcoal rot and soft rot were collected and subjected to tissue isolation technique. The fungus *Fusarium* sp. and *Macrophomina phaseolina* were isolated from the dry rot and charcoal rot infected potato tubers in potato dextrose agar medium whereas, bacterial pathogen *Erwinia carotovora* was isolated from soft rot infected potato tubers in nutrient agar medium.

The cultures of the purified isolates were maintained on Potato dextrose agar and Nutrient agar medium for fungal and bacterial pathogens respectively. The periodical sub-culturing and mass multiplication were done on the respective medium to keep the cultures fresh and were used throughout pathological investigations.

Symptomatology

The symptoms observed under natural condition are as follows:

Dry Rot

The symptoms of dry rot of potato consisted of circular or oval, light brown to purple, small independent lesions measured 2-5 mm in diameter around lenticels. As the disease progressed many of these lesions coalesced to form bigger size covering a major portion of the tuber surface and cavities were formed which become lined up with fluffy white to brown mycelium ultimately the entire tuber dried and becomes hard.

Charcoal Rot

Charcoal rot infected tubers developed black spots ranging from 2 to 8 mm in diameter surrounding the lenticels and eyes. As the disease advances, the tissue underneath the skin becomes uniformly black upto a depth of 2 to 5 mm.

Soft Rot

Soft rot infected tubers developed small areas around wounds and numerous small sunken areas measured 0.3 to 0.6 cm in diameter. Small sunken marked boundary was observed between the soft and sound tissues later on infected areas

developed more or less over the entire surface of the tubers. A clear amber liquid exuded from decayed areas with offensive sulfurous smell.

Similarly, Paharia (1960), O'Brien and Rich (1976), Pushkarnath (1976) and Rai (1979) observed the symptoms of above mentioned diseases under natural condition.

Pathogenicity

Pathogenicity Test for Dry Rot Under Laboratory Condition

Pathogenicity test of *Fusarium* sp. was confirmed on ten healthy tubers of variety Kufri Badshah by inserting mycelium through 2 mm minor injuries made with the help of sterilized knife. The inoculated tubers were kept at 28 ± 2°C and inspected daily for the appearance of the symptoms. Parallel controls were maintained where tubers were not inoculated. After 10th day of inoculation, the tubers showed the water soaked sunkened lesions. These lesions turned dry, enlarged and covered a major portion of the tubers which ultimately resulting in the shrinkage of tissues and formation of wrinkles on the affected areas. On cut, potato tubers showed dark brown internal tissues. On reisolation from infected tubers under inoculated tests revealed the presence of the same fungus (*Fusarium* sp.). However, symptoms did not develop in control tubers and remained healthy.

Similarly, Rai (1979 and 1982) proved the pathogenicity of dry rot under laboratory condition.

Pathogenicity Test for Dry Rot Under Pot Culture

Pathogenicity test of *Fusarium* sp. was also conducted by soil inoculation technique. The observations on the occurrence of disease were taken at the time of harvesting.

At the end of growing season, the plants along with tubers were dug out for symptom examination. All the tubers collected from inoculated pots showed typical symptoms of dry rot disease as the tuber surface turned brown to dark brown, circular or angular and sunken lesions were clearly seen. These necrotic lesions measuring upto 2.0 to 3.0 cm in diameter initially and the lesions also coalesced to form bigger patches covering the major portion of the tuber surface. At later stages, the old infection foci developed white star-like rays in the flesh visible through the skin and finally the entire tubers were hard and dry. The tubers of uninoculated control pots did not show any symptoms on tubers and remained healthy at harvest. The pathogen was reisolated from these dry rot affected potato tubers and compared with original one. Pure culture on reisolation resembled with the original isolate of *Fusarium* sp. Hence, pathogenicity was proved.

Similarly, Rai (1979) proved the pathogenicity of the dry rot pathogen of potato under pot culture.

Charcoal Rot: Healthy tubers of potato variety Kufri Badshah was tested in the laboratory condition by using the toothpick method of inoculation technique. The tubers initially showed the symptoms after 10 to 12 days in the form of black spots 2-8 mm in diameter. The skin of the tubers remains unaffected but the tuber flesh gets blackened to a depth of 2-5 mm. Fungal pathogen was reisolated and compared with the original isolate of *Macrophomina phaseolina*.

Similarly, Thirumalachar (1953), Sahai *et al.* (1970) and Upadhaya (1976) proved the pathogenicity of the charcoal rot pathogen of potato by toothpick method and causal organism was identified as *Macrophomina phaseolina*.

Soft Rot

1. Slice method: The bacterial pathogen *Erwinia carotovora* isolated from soft rot infected potato tubers were inoculated in the form of thick paste (10^8 cells) on the surface of healthy tuber slices. The inoculated slice samples developed softened, slimy, pulpy and watery mass accompained by production of characteristic odor after 24-48 h. This was taken as positive reaction for identification of *Erwinia carotovora* (Shashirekha *et al.,* 1987). Reisolation from infected tuber slices revealed the presence of the same bacterial pathogen while, control tuber slices remained healthy after 24 to 48 h.

Similarly, Shashirekha *et al.* (1987) and Arsenijevic *et al.* (1993) proved the pathogenicity of soft rot pathogen of potato.

2. Injection method: Tubers inoculated by injection as described in Material and methods were cut open after five days of incubation. The results are presented in Table 1.

3. Pin prick method: Tubers inoculated by pin prick method as described in Material and methods were also examined after five days of incubation. The amount of rotting was most consistent as it revealed from Table 1. Necessary checks were also maintained for each inoculation method and kept as control. The pathogen was reisolated from rotten tissue of potato from each method of inoculation and it was confirmed to be the presence of same bacterial pathogen as *E. carotovora*. Control tubers did not show any symptoms of the soft rot and remained healthy in each method of inoculation.

The results presented in Table 1 revealed that pin prick method and slice method were most consistent which showed uniform rotting, softening of slices / tubers with offensive foul smell from inoculated tubers / slices. However, the amount of rotting in case of pin prick method and slice method were significantly more as compared to injection method. In injection method of inoculation, the rotting was limited to the pulp near the point of inoculation.

Similarly, Henniger (1965) reported partial immersion of tuber tissue cylinders in soft rot bacterial suspension found more reliable with reproducible results as compared to injection method. Dorozhkin and Generalova (1981) reported that pricking of potato tubers and dipping in bacterial suspension found cheap and reliable method of tuber inoculation. Saini and Parashar (1981) tested pathogenicity of soft rot disease of potato by injecting (2.28×10^4 cells/mL) in sterilized potato tubers and incubated in desiccators at room temperature. They reported that *Erwinia carotovora* decayed the inoculated tuber tissues after 7 days of inoculation and the rotted tubers gave an offensive sulfurous smell. Kumar *et al.* (1992) used injection method, pin prick method, eye bud method, gunny bag rubbing method and slurry coating method for proving the pathogenicity of soft rot isolate and found that the amount of rotting in case of injection method was significantly more as compared to pin prick method. The present findings are in agreement with above research workers.

Morphology and Identification of the Pathogens

Morphological characters of the fungal pathogens were studied by observing slides, stained with cotton blue under a microscope.

Table 1: Amount of Rotting Observed in Tubers Inoculated by Different Methods of Inoculation

Inoculation method	Reuslts	Remarks
Slice method	Rotting ++++	Consistent, uniform rotting and softening of slice with foul odour.
Injection method	Rotting ++	Consistent, rotting limited to the pulp near the point of inoculation.
Pin prick method	Rotting ++++	Consistent, uniform rotting, clear amber liquid with offensive foul smell.
Check	No rotting	No rotting was observed.

Note: No. of '**+**' signifies the amount of rotting

Fusarium sp.

Colony: The fungal colony of *Fusarium* sp. was off-white with purple tinge, initially floccose later on changed into deep olive buff and finally becoming felted in old cultures.

Mycelium, conidia and chlamydospores: Mycelium branched, septate and inter and intracellular. Conidia both micro and macro, chlamydospores both intercalary and terminal abundantly found in one week old cultures. Microconidia were oval to cylindrical measuring 2-10 x 2-5 µm, macroconidia fusoid, curved to cylindrical, upto 7 septate measuring 8-40 x 2-10 µm and chlamydospores measured 2-18 µm in diameter.

Macrophomina phaseolina

Colony: Hyphae branched, septate and greyish white, dark grey, olivaceous green or brown. Mycelium sparse or fluffy. The fungus forms sclerotia in the culture media.

Sclerotia: The sclerotia were minute, black, smooth and measured 50 to 1000 µm in diameter. Sclerotia were formed by the interwining of the primary branches arose from the major hyphae. These cells enlarge become globose and turned cemented together by a gelatinous matrix.

Erwinia carotovora* subsp. *carotovora

Morphological characters of bacterial pathogen *Erwinia carotovora* were studied by preparing a smear of bacterial suspension of *E. carotovora* on the slide. The slide was then examined under oil immersion. Bacterial pathogen *E. carotovora* was non-spore forming, gram negative, short rod measured 1.5-3.0 µm long and 0.6-0.9 µm wide with rounded ends, occurring singly or in chains. It was motile with two to six peritrichous flagella.

The bacterial isolate (*E. carotovora*) was identified on the basis of morphological characters and confirmatory test by inducing the soft rot production in healthy tuber slices by inoculating 10^8 cells in the form of thick paste on surface of healthy tuber slices. Softening of slices accompanied by production of characteristic offensive odor was taken as positive reaction for identification of isolated bacterial as *E. carotovora* pathogen (Shashirekha *et al.,* 1987).

The identification of pure cultures of fungal pathogens was made on the basis of morphological characters of somatic and reproductive structures and was compared with those described in literatures. Thus, it was identified as *Fusarium* sp. and *Macrophomina phaseolina* causing dry rot and charcoal rot of potato, respectively. The pure cultures of fungal pathogens were also sent for identification to Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute,

New Delhi-110 012 (Reference No. 4187.2K and 4186.2K).

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