FURTHER STUDIES ON THE WHITE RUST OF BRASSICA


By

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DIVISION OF MYCOLOGY AND PLANT PATHOLOGY

INDIAN AGRICULTURAL RESEARCH INSTITUTE

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By

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CERTIFICATE

I hereby certify that the thesis entitled "Further studies on the white rust of Brassica caused by Albugo candida (Pers. ex. Lev.) Kunze" submitted in partial fulfilment of the requirement for the degree of DOCTOR OF PHILOSOPHY in MYCOLOGY AND PLANT PATHOLOGY, Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, is a record of bona fide research work carried out by Ms. Indrani Lahiri, under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance received during the course of this investigation has been duly acknowledged by the student.

New Delhi  

(Dr. T.P. Bhowmik)
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INTRODUCTION

Oilseeds occupy a significant place in India's national economy next only to cereals and pulses. Our country enjoys the unique distinction of growing a large variety of oilseed crops over a wide range of climate zones and also ranks third in the world in terms of area and production, Canada being the first and China the second.

Ironically, from a position of surplus in oilseed production about 25-30 years ago, the country is now facing an acute shortage of edible oil to meet the dietary demands of our growing population. In spite of several ameliorative steps taken up by the government in the recent years, there has only been marginal increase in their production. This is mainly due to the extension of their cultivation into new areas but their average productivity has not shown the desired rate of increase.

The area under oilseed has increased from 20.13 million hectares in 1987-88 to 21.64 million hectares in 1988-89 and production from 12.65 million tonnes to 17.89 million tonnes during the corresponding period (Anonymous, 1990). The per capita availability of edible oil in 1988-89 was about 6.5 kg, which is much below the desired nutritional standards (Vasudevan, 1990). For the eighth plan period the production target of oilseeds has been fixed to 22-24 million tonnes. Stepping up of productivity through removal of various production constraints will be necessary to realize the above objective.

Among the nine oilseed crops grown in this country, rapeseed and mustard rank second after groundnut. In 1988-89, they occupied an
area of 4.87 million hectares producing 4.41 million tonnes of oilseeds with a national average yield of only 905.5 kg/ha which is extremely low (Anonymous, 1990). The world average yield has been reported to be 1080 kg/ha (Anonymous, 1986b).

Out of the various oil yielding Brassicas that are commonly grown in India and other Asian countries, mustard (Brassica juncea Coss.) and rapeseed (Brassica campestris L.) are the most important. These are extensively grown during the rabi season in the Indo-gangetic alluvial soils of north and north-eastern states. They are grown in the states of Uttar Pradesh, Rajasthan, Madhya Pradesh, Assam, Haryana, Gujarat, West Bengal, Orissa, Punjab and Bihar, of which the state of Uttar Pradesh alone accounts for about 60 per cent of total cultivated area under the crop.

Among the various constraints that limit the productivity of rapeseed and mustard in India, the attack by Alternaria blight (Alternaria brassicae (Berk.) :Succ.) and white rust (Albugo candida (Pers. ex. Lev.) Kunze) are very serious and widely prevalent. Downy mildew (Peronospora parasitica (Pers. ex. Fr.) Fr.,) is yet another disease which is found in association with white rust and causes considerable damage.

With the extension of rapeseed and mustard cultivation into new areas, mostly with susceptible cultivars as monocrop and higher inputs of fertilizers and irrigation, white rust now appears in almost epiphytotic form causing serious loss in yield.
Although the pathogen *A. candida* received the attention of earlier Mycologists, white rust disease has not been studied in detail by past workers because of its sporadic occurrence and minor damage caused to the crop.

The present investigation was undertaken with the specific objective of obtaining information on the prevalence of biologic race(s) that parasitize rapeseed and mustard in India, establishment of dual culture of the pathogen and its host for maintenance and multiplication, changes in biochemical constituents of resistant and susceptible hosts, and formulation of an economic spray schedule for disease management. The results obtained are presented in the following pages.
Rape or mustard is an important oilseed crop of India, particularly in the northern states. The different types of oleiferous Brassicae grown in India are generally divided into three groups viz. rai (raya or laha), sarson and toria (lahi or maghi-lahi). Commonly rai or mustard refers to *Brassica juncea*, sarson to *Brassica campestris* var. sarson (yellow and brown) and toria to *B. campestris* var. toria. Besides these three main oleiferous Brassicae, two other species viz., *Brassica nigra* and *Brassica juncea* var. rugosa are found to a limited extent and are generally known as Banarasi rai and Pahadi rai respectively. *B. tournefortii* (Jangli rai) is another species which is cultivated to a limited extent (Singh, 1958).

According to Prain (1898) rai or mustard (*B. juncea*) originated in China which found its way into India through a north-eastern route. For brown sarson (*B. campestris* var. brown sarson) north western India is believed to be one of the independent centres of origin while north eastern India is the probable primary centre of origin of yellow sarson (*B. campestris* var. yellow sarson).

White rust disease of rapeseed and mustard is a common disease of all crucifers and is widely distributed throughout the country. From India the fungus was first reported by Butler (1918) and then by Mitter and Tandon (1930), Galloway (1935), Choudhary (1944), Ramakrishnan and Sundram (1952), and Safeeulla (1952) from different parts of the country. Other countries like Palestine (Rayas, 1938); Germany
(Klemm, 1938; Raabe, 1939); Burma (Seth, 1939); Brazil (Viegas and Teixira, 1943); France (Darpoux, 1946); Romania (Savulescu, 1946); Turkey (Bremer et al., 1947); Japan (Hirata, 1954); Canada (Greelman, 1963; Petrie, 1973); and Pakistan (Perwaiz et al., 1969) have also reported the disease.

Economic Importance

Earlier workers (Klemm, 1938; Chupp, 1925; Loof, 1960) reported that the disease causes little yield loss and is of slight economic importance on rapeseed. However, in recent years greater importance is being given to the disease because of its widespread occurrence resulting in substantial yield losses.

Mere localized infection on leaves may not result in much yield loss but maximum losses occur due to systemic infection of inflorescence resulting in their malformation (stagheads). Petrie and Vanterpool (1974) reported that systemic stem infection causes an average reduction of 60 per cent in seed yield on individual plants in Canada. In Saskatchewan (Canada), the average yield reduction in turnip rape (B. campestris) for the years 1970 to 1973 has been 6 per cent, ranging from 3 to 9 per cent (Petrie, 1973).

From India, Bains and Jhooty (1979) reported that B. juncea plants yield respectively 37 to 47 per cent and 17 to 32 per cent less siliquae and seeds due to mixed infection of white rust and downy mildew. Kolte and Tewari (1980) also noted 34 per cent loss in per plant seed yield of B. campestris var. Toria due to simultaneous
occurrence of white rust and downy mildew.

Symptoms

Symptoms of the disease appear on all above ground plant parts including the fruits, and infection may be both local as well as systemic (Butler, 1918; Walker, 1957). Butler, (1918) described the disease symptoms in detail. According to him, in case of local infection, symptoms of the disease appear on leaves and are characterized by the appearance of white or creamy yellow raised pustules (1 to 2 mm in diameter) which later coalesce to form patches. The pustules are scattered on the under surface of the leaves. The upper surface, corresponding to the pustule on the lower surface, is tan yellow and the prevalence of the disease is easily recognized from the upper surface of the affected leaves. After the complete development of the pustule, it ruptures and releases a chalky dust of spores (sporangia). Necrosis of the leaf tissue is not observed immediately with the appearance of pustule. When the white rust pustule ages and the affected leaves are about to become senescent, the necrosis around or in the pustule may be seen. Thickening or hypertrophy of the affected leaves is usually not seen in rapeseed and mustard. In some other crucifers, however, affected leaves have been reported to show hypertrophy (Mundkur, 1959). Systemic infections are manifested by distortion, hypertrophy, hyperplasia and sterility of inflorescence often referred to as the staghead (Petrie, 1973). The distortion and abnormal development are particularly conspicuous in flowers. Sepals become enlarged to several times the normal size, petals enlarge and turn green, while the stamens are
Plate 1. White rust infected leaf of cv. Pusa bold.

Plate 2. White rust infected inflorescence of cv. Pusa bold. Note the hypertrophied condition of floral parts as compared to adjacent healthy flower.
transformed into leaf like or carpelloid structures. The petals and stamens persist in the flower instead of falling early as in the case of normal plants. The stamens are sometimes changed into thick club shaped sterile bodies. The ovules and pollen grains are usually atrophied, resulting in complete sterility.

Drying of severely affected plants is due to imbalance of nutrition between the host and parasite (Vasudeva, 1958). Symptoms on the roots are rarely seen. However, Loubert (1932) reported the presence of puffy fleshy swellings on the upper part of the tap root of radish. Root galls containing oospores of *A. candida* were also reported on long scarlet radish by Fischer (1954).

**Fungi associated with white rust**

Downy mildew and Alternaria blight are two diseases which occur along with white rust disease on Brassica plants. According to Butler (1918) downy mildew (*Peronospora parasitica* (Pers. ex. Fr.) Fr.) is frequently found in coexistence with *Cystopus candidus* (*A. candida*) on cruciferous hosts. It is not easy to separate their effects, but *Peronospora* produces the greatest deformities in the stem, *Cystopus* in flowers. The axis of the inflorescence is equally liable to deformity. The leaves and flowers are not often swollen, except the young ovary. The floral buds are atrophied, all the parts, sepals, stamens and pistil, being shrunken and almost colourless. There is never any trace of violet colour produced so often by *Cystopus* (*Albugo)*.

Under natural conditions, mixed symptoms of downy mildew
and white rust are usually seen on the same inflorescence (Bains and Jhooty, 1979; Kolte and Tewari, 1980).

According to Chaurasia et al. (1982) *P. parasitica* and *A. cruciferarum* SF Gray (*A. candida*) respectively incite downy mildew and white rust on the leaves and other aerial parts of mustard (*Brassica campestris*) plants. The downy mildew infection is rare on leaves but remains confined mostly to the galls caused by the oospore stage of *A. cruciferarum*. Cross sections of the galls and galls infected with *P. parasitica* showed oospores. The morphological characters of oospore in both cases were similar to those of *A. cruciferarum* oospores. This indicates that the galls were produced by the systemic infection of *A. cruciferarum* in *B. campestris* although *P. parasitica* also causes galls in mustard plants when it is systemic. However *P. parasitica* galls are mostly produced on floral parts.

On the leaves the downy mildew symptoms are found associated with white rust symptoms. Bains and Jhooty (1984) observed that *P. parasitica* appeared in and/or around the white rust pustules on leaves and inflorescence of mustard plants but when the downy mildew lesions appeared first on leaves, the appearance of white rust pustule in or around the downy mildew lesion was seldom seen.

Another common associate of white rust disease is *Alternaria* blight. Three species of *Alternaria* viz., *A. brassicae* (Berk) Sacc., *A. brassicicola* (Schw) Wilts, and *A. raphani* Groves and Skolko have been found to affect the rapeseed and mustard crop quite commonly throughout the world. The disease caused by *A. brassicae* is more
destructive and occurs more frequently than the one caused by either of the other two species.

Petrie and Vanterpool (1974) reported over 20 species of fungi including several pathogens of crucifers in association with hypertrophies of the inflorescence, stem and pod blisters produced on turnip rape (B. campestris), wild mustard (B. kaber) and false-flax (Comelina microcarpa) by the white rust fungus, A. cruciferarum. The most prevalent associates of Albugo were P. parasitica, Alternaria alternata, Fusarium roseum 'Accuminatum'... and 'Equiseti', A. raphani, A. brassicae and Cladosporium species.

The organism

Albugo candida (Pers. ex. Lev.) Kunze, the causal organism of white rust belongs to Family Albuginaceae of the order Peronosporales. The literature regarding the nomenclature of the fungus has been reviewed by Verma (1987).

The genus Albugo, comprises thirty species of biotrophic parasites, which are host family specific. Mycelium is aseptate, intercellular, widespread, particularly in leaves, 4-12 μ in diameter, hyaline, thin walled, bearing simple globose intercellular haustoria. Haustorial body lacks nuclei. The fine structure of the haustorium has been studied by Berlin and Bowen (1964). Webster (1980) described the mycelium of Albugo to penetrate the tissues of the plant in intercellular spaces, small lobular haustoria were produced frequently which penetrated the wall of the parenchymatous cell of the host. The intercellular
mycelium massed beneath the host epidermis to form a palisade of cylindrical sporangiophores which gave rise to chain of spherical sporangia in basipetal succession; pressure of developing chains of sporangia ruptures the host epidermis.

Sporangiophores, crowded into sori, clavate, hyaline, are 14-44 x 8-18 μ, average 28 x 13 μ in size. Sporangia are joined by coloured connectives (disjunctors) in basipetal succession into chains, all alike, hyaline, globose, elliptical or truncated, obovate, thin walled, without annular thickened band and are 12-23 x 12-20 μ, average 17 x 16 μ in size.

Sporangium usually germinates by giving rise to zoospores and very rarely directly by the germtube. Formation of zoospores was first reported by Prevost (1807). Both Tulasne (1854) and Hoffman (1859) observed germination by germtubes. However, deBary in 1860, gave details of zoospore formation and its structure. Zoospores are slightly concave, biflagellate with one short and one long flagella.

Sporangia of Albugo do not germinate except in the presence of free moisture. Edie and Ho (1970) observed that sporangia of A. ipomoeae aquaticae did not germinate in the absence of free water even when they were maintained for several days at optimum temperature and in atmosphere as near as possible to 100 per cent relative humidity.

Melhus (1911) reported sporangial germination of A. candida to occur between 0 to 25°C and believed the optimum to be near 10°C. Perham (1942) noted 13°C as optimum for their germination.
Takeshita (1954), working on *A. candida* on horseradish, reported 10 to 15°C as the most favourable temperature for indirect germination of sporangia and 15 to 20°C for germtube elongation. Endo and Linn (1960) found 15 to 20°C as the overall optimum for germination.

Eberhardt (1904) stated that sporangia from pustules just ready to open germinated best. Melhus (1911) on the contrary, failed to germinate the sporangia from just opened pustules. Edie and Ho (1970) showed that immature sporangia from young unopened pustules, failed to germinate while mature sporangia from pustules, just after natural rupture, or the ones fallen in masses of white powder gave best germination.

Wagger (1886) and Webster (1980) observed numerous sexual organs in intercellular spaces in hypertrophied tissues of stem and leaves. Sex organs are formed from the mycelium in the intercellular spaces, particularly in the systemically affected plant tissues. Oogonia are borne singly on short terminal or lateral branches, globose about 40 μ diameter; antheridia are club shaped, 16-24 x 24-32 μ, arising near the oogonia. Detailed studies on the reproduction of *A. candida* was made by Wagger (1886). Oospores formed are globose, dark brown, 40-60 μ, average 50 μ in size, highly differentiated with 5 layered cell wall. Epispore is 4-7 μ thick, tuberculate with flattened branched ridges.

**Host range**

*Albugo candida* (Pers. ex. Lev.) Kunze occurs on all members of Cruciferae and also some species of Capparidaceae and Cleomaceae
(Walker, 1957). Parisi (1924) reported the fungus on non-cruciferous host
Onobrychis crista, the first record of this genus on Papilionaceae.
The fungus was determined as a strain of A. candida to which the name
variety "mauginii" was given. Wiese (1927), found it on wall flowers
(Cheiranthus cheiri) and on Malhiola incana in association with
Peronospora parasitica. According to Togashi et al. (1930), the
fungus occurs on 118 species belonging to 42 genera of Cruciferae.
It is also known to attack Cardamine subumbelata (Damle, 1944);
Camelina sativa (Wahlin, 1951); S. pinnatifidum (Pelluchi, 1951) and
Aubrietta spp. (Henderson, 1955). Baker (1955) reported it on Brassica
campestris L., B. oleracea L. var. botrytis, B. rapa L., Capsella
bursapastoris L., Cardamine hirsuta L., Cleome spinosa L., Lepidium
oleraceum Frost, L. ruderale L., L. sativum L., Malcomia maritima
R. Br., Raphanus sativus L., Rotippa islandica Oeder and
Sisymbrium officinale L. It is less commonly found on cabbage, brussels-
sprouts and cauliflower than on radish, horseradish, turnip, rapeseed and
mustard (Chupp and Sherff, 1960).

Biological specialization

Biological specialization was first noted by Eberhardt (1904)
who recognized two specialized groupings of the fungus: one attacking
Capsella, Lepidium, and Arabis, the other attacking Brassica, Sinapis
and Diplotaxis. He thought that conidial measurements were too variable
for taxonomic purposes. Melhus (1911) noted host specificity in collections
from Lepidium, Raphanus, and Capsella from Wisconsin. Similarly
Hiura (1930) studied samples from Brassica, Capsella and Raphanus
in Japan. On the basis of host specialization, Napper (1933) described
20 races of *A. candida* in Britain. Conidial measurements of specific races on *Raphanus, Brassica, Capsella, Cardamine, Draba, Arabis* and *Armoracia* led Togashi and Shibasaki (1934) to propose the two intraspecific morphologic taxa *A. candida macrospora* and *A. candida microspora*.

Other reports of host specialization are of a race on *Capsella* by Pape and Rabbas (1920) and one on *Armoracia rusticana* by Endo and Linn (1960).

In his monograph, Biga (1955) reviewed the early nomenclature of *A. candida* and listed 241 species in 63 genera of crucifers that are attacked. He recognized the two intraspecific morphologic taxa established by Togashi and Shibasaki in 1934 but renamed *A. candida microspora*, as *A. candida candida*. On the basis of conidial measurements from 63 species, Biga reported that *A. candida macrospora* (average diameter of conidia=15-17.5 µ) was restricted to *Armoracia, Brassica, Erucastrum, Raphanus*, and *Rapistrum* whereas *A. candida candida* (average diameter of conidia = 12.5 - 15 µ) had a wide range of cruciferous hosts. Further, conidial size and host specialization were related on a broad basis.

A review of this literature reveals that the authors were hesitant to describe specialized races of *A. candida*. Most of the work does not reveal the clear-cut positive or negative sporulation responses characteristically found, for example, in wheat populations attacked by the Uredinales.

Pound and Williams (1963) were the first to establish a firm
basis for classifying biologic forms of *A. candida*. Collections of *A. candida* from six different cruciferous hosts were cross-inoculated on a number of wild and cultivated Crucifer species. Each collection exhibited a different host range and was classified as a distinct biological race of the fungus. In every case, the original host species from which the isolate was taken was the best differential host for that race. The races were described as follow:

Race 1 from *Raphanus sativus* var. Early Scarlet Globe, race 2 from *Brassica juncea* var. Southern Giant Curled, race 3 from *Armoracia rusticana* var. Common, race 4 from *Capsella bursa-pastoris*, race 5 from *Sisymbrium officinale*, race 6 from *Rorippa islandica*. Average lengths of 100 conidia from each race on their natural hosts were as follow:

(A. candida macrospora); race 1, 16.8 μ; race 2, 17.7 μ; race 3, 17.5 μ; race 6, 17.5 μ; (A. candida candida); race 4, 15.2 μ; race 5, 15.5 μ. Conidial sizes of a given race varied considerably on certain non differential hosts.

Later race 7 was reported on *B. campestris* L. (Verma et al., 1975) and race 8 on *B. nigra* (L.) Koch (Black mustard) (Delwiche and Williams, 1977).

Petrie (1988) studied the races of *Albugo candida* on cultivated cruciferae in Saskatchewan. According to him white rust from *Raphanus sativus, Brassica juncea, Capsella bursa-pastoris* and *B. campestris* represented 4 distinct races of *A. candida*. Races were differentiated on *R. sativus, B. juncea* cv. Southern giant curled,
Capsella bursa pastoris and B. campestris subsp. pekinensis cultivars. Some sarson accessions (B. campestris subsp. sarson) were susceptible to both B. juncea and B. campestris races. The radish collection, lightly infected a number of B. campestris breeding lines and upto 30 per cent of the plants in a few older B. juncea cultivars. The collection from Capsella did not infect any of the Brassica cultivars tested. The B. juncea race infected less than 10 per cent of the plants of B. campestris cultivars torch and tobin but infected upto 35 per cent of plants in cv. candle and 59 per cent of the plants in a B. campestris breeding line from Finland. B. napus (rape) and rutabaga were highly resistant to all the races. A few yellow sarson accessions from Nepal were resistant to both the B. campestris and B. juncea races and are important from a resistance breeding perspective.

Dual culture of obligate parasite and host using tissue culture technique

The term tissue culture, in its widest sense can be used to describe the growth of isolated plant organs (roots, shoot tips, embryos etc.) anthers and pollen etc. in culture on defined and semi-defined media, in the absence of contaminant microorganisms. The techniques involved have recently advanced considerably, particularly in the development of desired media, and although the classical works of White (1954) and Gautheret (1959) remain useful sources of information, practical guidance on modern methods and procedures have been described by Willmer (1966), Street (1973 and 1974), Cruse and Patterson (1973), and Gamborg and Wetter (1975), Bhojwani and Razdan, (1983) and Dixon (1985).
The application of tissue culture technique in plant pathology have been reviewed by Braun and Lipetz (1966), White (1968), Maheshwari (1969), Ingram (1973) and Ingram and Helgeson (1980). Plant tissue culture techniques were originally developed for the purpose of studying fundamental problems of nutrition and morphogenesis and have made important contributions in these fields. In addition, it has become increasingly clear that cells and tissues, cultured under controlled aseptic conditions, have an important role to play in other branches of plant biology including pathology. They have already been extensively employed in studies on the crown gall disease caused by *Agrobacterium tumefaciens* (Butcher, 1973) and their potential uses in the study of other plant diseases are being explored and developed (Ingram, 1973). Ingram and Helgeson (1980) reviewed the use of tissue culture technique for the growth and study of obligate fungal parasites.

The possibility that a dual culture consisting of an obligate or near obligate fungal biotroph and its host might provide a simplified experimental system for studies of the interaction between the two organisms was first realized by Morel (1944, 1948).

In a series of pioneering experiments, Morel successfully inoculated callus tissue cultures of *Vitis vinifera* with aseptic zoospores of the downy mildew fungus *Plasmopara viticola* and was able to maintain the resulting dual cultures for extended periods by subculture and reinoculation; aseptic sporangia produced on the dual cultures were subsequently used in fungicide tests. Morel (1944, 1948) surface sterilized infected vine leaves and allowed the fungus to grow
out through the leaf surface again. Thus, having removed spores aseptically in sterile distilled water, he allowed zoospores to form and used these to inoculate vine callus cultures. A reasonable number of uncontaminated cultures were obtained in this way.

Since the experiments conducted by Morel, several other dual cultures of fungal biotrophs and their hosts have been established (Ingram, 1973), although the number of species involved is remarkably small and few have been investigated physiologically. Also various techniques have been used to bring about host parasite combinations.

Aseptic spores of fungi which cannot be grown in axenic culture can usually be obtained by surface sterilizing infected organs and then allowing the fungus to sporulate in a sterile chamber. This technique, variously modified, may be used successfully with many members of the Peronosporales and Uredinales. Successful infection of host callus cultures by placing aseptic spores or mycelium directly on the tissue surface has been achieved with a number of species. Ingram and Joachim (1971) obtained aseptic inoculum of *Peronospora farinosa* by infecting detached pairs of cotyledons from 7 to 14 days old seedlings of a susceptible sugar beet cultivar with a suspension of conidia of *P. farinosa* from infected sugar beet plants.

Initiation of dual cultures was done by transferring uncontaminated cotyledons showing fungal sporulation aseptically on to the surface of established host callus culture. Incubation was done at 15°C at low intensity white light. In most cases the fungus spread from the cotyledons and became established in the callus tissue within
10 days. Uncontaminated sporulating cotyledons could also be placed directly on the callus tissue culture media. Callus was formed at the cut surfaces and in many cases contained systemic mycelium.

Methods similar to the one used in this case have provided contaminant free spores of *Peronospora parasitica* (Ingram, 1969c) and *Bremia lactucae* (Mason, 1973).

According to Webb and Gray (1980), in case of the Erysiphales, surface sterilization may be inappropriate because of the superficial habit of the mycelium. In such a case non sterile spores may be transferred through a succession of aseptic plants until unwanted microorganisms are diluted out. They maintained *Erysiphe pisi* and *Erysiphe graminis* f.sp. *tritici* inoculum by shaking old infected leaves over healthy plants every three weeks. Aseptic pure culture of these were obtained by successive transfer of a few conidia onto surface sterilized detached leaves in culture.

The difficulty of infecting callus tissues with spores of some fungi has led to the initiation of dual cultures by incubating pieces of surface sterilized, systemically infected host tissue on a suitable culture medium. This technique was first used with *Gymnosporangium juniperivirgininae* which causes telial galls on *Juniperus virginiana*, (Hotson and Cutter, 1951) and has since been used with other host and parasite combinations, notably *Uromyces ari triphylli* and *Arisaema triphyllum*. (Cutter, 1960), *Plasmodiophora brassicae* and *Brassica* spp. (Ingram, 1969a) and *Cronartium ribicola* and *Pinus monticola*. (Harvey and Grasham, 1970).
Griffin and Coley Smith (1968) obtained contaminant free sporangia of *Pseudoperonospora humuli* from surface sterilized segments of systemically infected hop stems. The incidence of bacterial contamination in these segments was usually less than 50 per cent. Tiwari and Arya (1969) infected callus tissues of pearl millet with *Sclerospora graminicola* by placing on them small fragments of systemically infected flower tissue aseptically dissected from the apical region of a mature infected plant.

Ingram (1968a) initiated infected callus cultures from *Plasmodiophora brassicae* clubs from 4 to 6 week old plants. Sterilized systemically infected clubs were grown in the tissue culture media. Degeneration of the callus followed as resting spores were formed. On suitable media it was found that *Plasmodiophora brassicae* may be maintained in complete balance with host callus.

Hardev Singh (1963) was the first to report growth of a member of the Albuginaceae in the tissue culture of its host. He established *Albugo ipomoeae-pandurate* in association with *Ipomoea pentaphylla*. Hypertrophied stems, petioles and flowers forming galls systemically infected with *Albugo ipomoeae-pandurate* were used. Small sterilized portions of the galls were inoculated on the appropriate tissue culture medium. The gall tissue proliferated to form callus which was maintained for over a year by subculturing at intervals of less than two months. In about 30 per cent of the cultures, after they had been growing for 40 days on a fresh medium, the white globules showed numerous long chains of conidia coming out of the surface. A
histological examination showed a coenocytic mycelium freely proliferating in the intercellular spaces in the callus in about 80 per cent of culture tubes. Small round haustoria were present in abundance inside the host cells. Sixty per cent of the cultures showed an abundance of oospores which fully resembled those occurring in nature. Unfertilized oogonia were also observed at the surface of the callus. For the first three weeks after inoculation on a fresh medium a callus with oospores grows at a much faster rate as compared to the one without them. In about 20 per cent of the cultures the fungus showed only the vegetative phase. Some of the explants became free of the fungus after repeated subculturing. Such a callus was visibly different from an infected one.

Balance in culture

Following initiation of dual cultures, continued growth is dependent upon the establishment of a balanced relationship between the component organisms comparable with that existing in nature. This is not always possible (Brian, 1967) although in some instances the natural equilibrium between host and parasite may not only be retained in culture but enhanced. It is perhaps not surprising that most of the combinations which can so far be placed in this category involve fungi which have a systemic growth habit and cause morphological disturbance in the host plant, e.g. Sclerospora graminicola, Peronospora farinosa, Gymnosporangium juniperi-virginianae, Cronartium ribicola and Plasmodiophora brassicae (Ingram, 1973). Of these combinations only that involving Plasmodiophora brassicae has been investigated in detail. It has been shown that Brassica callus tissues infected with
secondary plasmodia of *P. brassicae* proliferate more rapidly than healthy tissues, and are even able to grow in the absence of exogenous cytokinin and auxin (Ingram, 1969a and b; Williams *et al.*, 1969; Dekhuijzen and Overleem, 1971).

The ability to live in balance with host tissue cultures differs with different host-fungus associations. For example, while the dual cultures of sugar beet and *P. farinosa* live in complete harmony, the callus tissues of *Brassica* species are killed within 7 to 14 days of infection with *P. parasitica* (Ingram, 1969c). Axenic combination of *Peronospora parasitica* and callus of *Brassica oleracea* var. *capitata* (drumhead cabbage) and other *Brassica* spp. were established. The fungus grew well in callus culture and sometimes had many of the characteristics of an aggressive parasite in contrast with its growth habit in intact cotyledons of *Brassica* spp. The dual cultures could only be maintained by inoculating non infected host callus cultures with explants of tissue from moribund dual cultures at regular intervals.

In some instances the reverse situation may be obtained with a vigorous host tissue outgrowing the fungus. This was sometimes the case with dual cultures of sugar beet with *P. farinosa* grown on the defined culture medium (Ingram and Joachim, 1971).

The balance between fungus and its host in culture is likely to be influenced by the degree of biotrophy inherent in the fungus and by the relative vigour of the two organisms. The latter factor may be susceptible to modification by variation in the physical components of the culture environment such as the levels of growth substances.
Biochemical nature of disease resistance

Role of sugars

Horsfall and Dimond (1957), categorised a 'high sugar disease' and 'low sugar disease' based on sugar content and the vulnerability of the plant to become diseased. Powdery mildew and rusts are categorised as 'high sugar diseases' because the host is susceptible to the pathogen when its tissue contains high level of sugar. Plants become resistant when sugar content of their tissues falls below a certain level needed by the pathogen. In case of low sugar disease, the converse situation is encountered.

The role of sugars in plants in relation to their disease reaction has been studied by several workers (Lyles et al., 1959; Krog et al., 1961; Sindbad, 1978)

Prasad (1983) studied the free sugar content of coriander infected with stem gall disease and reported a decrease in its concentration as a result of infection. Reduction in reducing sugars and starch in white rust infected leaves of Boerhavia diffusa was reported by Kumar and Manoharachary (1985). Singh et al. (1987) observed higher reducing sugar content in potato varieties of most susceptible Alternaria alternata, which decreased with the increase in resistance of the varieties.

Verma (1987) worked on the white rust disease of Brassica spp. According to her the concentration of reducing and non reducing
sugars was highest in the immune cultivar, followed in decreasing order of concentration by the moderately resistant and the susceptible cultivars. While rust infection caused reduction in their concentration in plants, the extent of reduction being significantly more in the susceptible cultivar than in the moderately resistant one. White rust disease of rapeseed and mustard may, therefore, be called a 'low sugar disease'.

Similar results were reported by Prasad (1990) while studying the sugar content in leaves of groundnut varieties infected with *Alternaria alternata*. He observed that total and reducing sugar contents were significantly more in healthy leaves of resistant and moderately resistant varieties than the highly susceptible ones, but due to infection of *A. alternata*, they were reduced significantly over healthy leaves. The reduction in percentage of total (reducing and non reducing) sugars was more in highly susceptible varieties in comparison to resistant and moderately resistant varieties.

Lukose *et al.* (1990) worked on the biochemical alterations in infected bajra leaves due to infection by a number of fungal pathogens. Results obtained by them too showed similar trends. Reduction in the level of sugar during pathogenesis was also observed by Narula and Mehrotra (1990) while studying biochemical changes in colocasia leaf tissues infected with *Phytophthora colocasiae*.

However, Maheshwari *et al.* (1985) found an increase in the synthesis of carbohydrates due to infection of *Brassica juncea* inflorescence by *Albugo candida*. 
Role of phenols

Phenolic compounds and related oxidative enzymes are generally considered as one of the most important biochemical parameters for disease resistance. A wide variety of phenolic compounds occurring in plants may often provide a partial explanation for the specificity of host parasite interaction. For example, certain plant species might contain or produce phenolic substances toxic to a particular pathogen whereas others (susceptible) may lack them (Kuc, 1963).

Kiraly and Furka (1962) found that resistance of Khapli wheat to most stem rust races was due to high level of phenols, against low endogenous phenol content found in susceptible varieties. Gangopadhyaya and Wylie (1973) found an inverse correlation between the total phenol content and susceptibility of soybean varieties to infection by *Macrophomina phaseolina*. Arora and Wagle (1985) working on wheat resistance to loose smut, and Bashan (1986) working on cotton seedlings, resistant and susceptible to *Alternaria macrospora* also reported that accumulation of total phenols is usually higher in resistant genotypes as compared to susceptible ones. Similar observations were made by Jayarat et al. (1990) while working with stem rot of rice caused by *Sclerotium oryzae*.

Several workers have investigated the appearance and accumulation of phenolic compounds in plant tissues in response to infection.

Chandramohan et al. (1967) showed that varieties of
Amaranthus tricolor resistant to Alternaria spp. accumulate orthodihydroxy phenol after infection and the amount was many times more than that in susceptible varieties. The total phenols and phenol oxidase activity increased in both resistant and susceptible cultivars after infection, but the increase was more in resistant cultivars.

Sridhar and Ou (1974) investigated the biochemical changes associated with development of resistant and susceptible types of rice blast lesions. They observed an increase in phenol content after infection. Similar observations were made by Sharma et al. (1983) while working on leaf blight of maize caused by Drechslera state of Cochliobolus heterostrophus.

Narula and Mehrotra (1990) also reported that phenols were more in leaf tissues of colocasia infected with Phytophthora colocasiae. Lukose et al. (1990) confirmed such an increase in phenol content while working with blast, leaf spot, rust and downy mildew of bajra.

However, contrary to the above reports, steady decrease in total phenols was observed due to infection by Sindha et al. (1978) while working with rust of sunflower. Rao and Dua (1978) also found decrease in total phenols in Amaranthus leaves infected with Albugo bilti as compared to the healthy ones, although the pattern was just the opposite for orthodihydroxy phenols. Dhingra et al. (1982) reported significant reduction in total phenolics in floral parts of Brassica campestris infected by A. candida. Gupta et al. (1990) also observed higher concentration of total phenols in mustard cultivars tolerant to Alternaria leaf blight as compared to susceptible ones at all stages of plant development but
after infection, their amount decreased in all the genotypes.

**Role of amino acids**

Presence or excess of certain amino acids are reported to induce resistance. Certain amino acid analogues when given to the host, inhibit fungal infection as has been observed in wheat stem rust (Samborski and Forsyth, 1960) and bean rust, *Uromyces phaseoli* (Poszen et al., 1966). These authors also found that an excess of a particular amino acid, such as D serine, DL-histidine, methionine and isoleucine are able to induce resistance. Purkayastha and Chattopadhaya (1975) reported comparatively higher contents of amino acids and amides in the Benibhog variety of rice than in Padma, a variety moderately resistant to *Helminthosporium* infection.

Verma (1987) working on the biochemical changes induced in *Brassica* spp. due to infection by *Albugo candida* showed that the susceptible cultivar had the highest concentration of total free amino acids followed in decreasing order of concentration by the immune and the moderately resistant cultivars. After infection with white rust, their concentration increased in both the susceptible and the moderately resistant cultivars with the progress of the disease, but the increase was significantly higher in the former as compared to that in the latter.

However, Prasad (1983) observed a decrease in amino acid content as a result of infection of coriander by stem gall disease. Apet and Suryawanshi (1990) estimated the total amino acid changes in leaves of groundnut cultivars resistant and susceptible to *Puccinia arachidi*. Although they found a higher concentration of total amino acids in the
susceptible cultivar as compared to the resistant one, there was a reduction in amino acid content as a result of infection. The reduction in the susceptible cultivar was 58 per cent over the uninoculated control while in resistant cultivars changes in amino acid content were practically negligible.

With a view to ascertain the biochemical nature of resistance, the concentration of free amino acid in cotton varieties, resistant and susceptible to *Myrothecium* leaf spot, was estimated by Taneja *et al.* (1990). They found, variations in the amino acid composition. The presence of six amino acids in healthy leaves of susceptible cultivars of cotton and their absence in infected leaves clearly showed that these amino acids are effectively utilized by the fungus during the process of pathogenesis. The absence of the six amino acids and the presence of leucine and tryptophan in the resistant varieties may be responsible for imparting resistance against this pathogen. Kamlesh Kumari *et al.* (1970) reported significant changes in amino acid content in hypertrophied inflorescence of mustard due to *A. candida* infection.

Conversely, Naik and Addy (1973) working on groundnut infected by *Cercospora personata* (*Cercosporidium personatum*) did not find any correlation between the free amino acid content and infection of the plant. No quantitative or qualitative differences between resistant and susceptible cultivars in respect to their free amino acid content was observed.

**Role of proteins**

Changes in protein metabolism in plant tissues may occur
during the initiation of infection and course of disease development. Dhingra et al. (1982) studied the biochemical changes in the floral parts of *Brassica campestris* infected by *Albugo candida* and reported that the amount of total proteins decreased significantly in all the infected floral parts and floral axis. Verma (1987) observed a decline in soluble proteins as a result of white rust infection on *Brassica* spp. and the decline was maximum in leaf followed in order of their reduction by inflorescence and stem.

However, observations of Maheshwari et al. (1985) were contrary to that stated above. They too worked on *Brassica juncea* inflorescence infected with *A. candida* and reported more concentration of proteins in the hypertrophied inflorescence axis. Narula and Mehrotra (1990) observed increased quantity of total soluble proteins in *Phytophthora colocasiae* infected leaf tissues as compared to its healthy counterpart.

Role of total nitrogen

Investigations have shown the existence of a relationship between the resistance of the host tissues and their total nitrogen content. Rao and Dua (1978) found an increase in both total nitrogen and amino nitrogen contents in *Albugo bilti* infected Amaranthus leaves. However, Verma (1987) reported that the concentration of total nitrogen registered a decrease in Brassica tissues following infection with *Albugo candida* but the decrease was of higher order in the susceptible than in the moderately resistant cultivar. Prasad et al. (1989) working on coriander infected with *Protomyces macrosorus* also observed a reduction in total nitrogen as a result of infection.
Chemical control

Field trials with a number of fungicides have been conducted in order to assess their suitability for the control of white rust and *Alternaria* leaf blight of mustard. Hämmerlund (1954) found that four applications of captan, zineb and Bordeaux mixture are effective against white rust on radish. On the other hand, *A. tragopogonis* though difficult to control, responded to some extent to treatment with maneb when applied frequently (Say, 1963). Stone and Dueck (1977) noted that seed treatment and foliar sprays of rapeseed and mustard with CGA 48988 prevent staghead production by white rust. According to Gupta and Sharma (1978), spray application of 0.2 per cent captafol, 0.2 per cent benomyl or 0.3 per cent Miltox proved effective against white rust and resulted in increased seed yield. In field trials, they obtained excellent control by Thiovit and Difolatan against foliar infection. Two applications of protectant fungicides, Bravo, Manzate 200 and DPX 164 reduce foliar infection of turnip rape by *A. candida* (Dueck and Stone, 1979). But the fungicides had no apparent effect on staghead formation.

Verma and Petrie (1979) reported the efficacy of 27 fungicides against white rust of turnip rape *in vitro* as well as in growth chamber tests under artificial inoculation. They obtained 75 per cent inhibition of oospore germination by mersil, PMA 10, at 500 ppm. Out of the nonmercurials tried, mancozeb and ethazole were the best, giving 60 per cent inhibition. In growth chamber tests under artificial inoculation, application of either chlorothalonil or mancozeb at 250 and
500 ppm respectively, both 6 hours and 1 week before inoculation, gave good control of white rust. Spraying of either of the fungicides both 24 hours and 1 week after inoculation was not effective. Two foliar sprays of chlorothalonil in June, significantly reduced both foliar and systemic infections. However, in view of the results of their growth room studies, they suggested a third application on turnip rape at the time of flowering.

Bains and Jhooty (1979) reported Blitox and Dithane Z-78 as effective against mixed infection of *A. candida* and *Peronospora parasitica* on *B. juncea*. Sharma and Sohi (1982) conducted trials with 10 fungicides against *Peronospora parasitica* and *A. candida* and showed that leaf spraying was more effective than seed treatment. Four sprays with Difolatan (Captafol) (0.3 per cent), Deconil (chlorothalonil) (0.1 per cent), Dithane M-45 (mancozeb) (0.2 per cent), Ridomil (0.1 per cent) and Aliette (0.1 per cent) at intervals of 8-10 days were found most effective in controlling both the disease and in increasing yield. Chen *et al.* (1984) applied two sprays with Rldomil in the growing season which gave 90-97 per cent control of white rust on *Ipomoea aquatica*.

Bhownik and Singh (1984) treated rapeseed and mustard seeds with Metalaxyl-35 (Apron) and reported that it causes no phytotoxic effect on the seedlings but sowing of such seeds proved ineffective in providing protection to the plants later against white rust under field conditions. Metalaxyl-25 (Ridomil-25) showed complete compatibility with captafol (fungicide) and metasystox (insecticide), the chemicals respectively known to be effective against leaf blight (*A. brassicae*) and aphid
(Lipaphis erysimi) of rapeseed and mustard. Application of five sprays at 15 days intervals starting from one month old crop with metalaxyl-25 either alone or in combination with captafol or with captafol and metasystox provided significant reduction in intensity, respectively of white rust and leaf blight, and of these two disease and aphid infestation besides increased seed yield. Saharan et al. (1984), used Ridomil (metalaxyl), pervicur and MB 21914 C against white rust of rape seed and mustard and reported that application of Ridomil (0.2 per cent) reduced leaf infection by 49.7 per cent, floral infection by 79 per cent and resulted in highest yield. It was followed by MB 21914C and pervicur in order of efficacy.

According to Velsteke and Meeus (1985), three or four sprays with triadimefon + chlorothalonil or tridomorph + fentin hydroxide also controlled A. tragopogonis. Dainello and Jones (1986) found the soil and seed treatments with metalaxyl are quite effective against white rust (A. occidentalis) of spinach. Application of metalaxyl at 0.1-3.7 Kg ai/ha in furrows as granules or to the soil surface as a bed spray checked white rust of spinach upto 48-60 days after planting, while seed treatment with metalaxyl 0.5-1.0 g ai/kg seed prevents its attack for 30 days.

Oxadixyl is an excellent systemic fungicide and is suggested for the control of downy mildew on grapes, blight caused by Phytophthora infestans on potato and tomato, and other diseases caused by fungi belonging to Peronosporales. Rossignol (1988) used new oxadixyl based mixtures for broad spectrum disease control of pea seeds. These gave
varying control of downy mildew, foot rot, leaf spot and damping off of pea. Mathur and Bhatnagar (1988) tested 6 fungicides as seed dressing for the control of mixed infection of white rust and downy mildew of mustard and reported Apron 35 SD and Readymix ZM to be the most effective. For management of Alternaria blight, Shivpuri et al. (1988) reported that Rovral was significantly superior to five other fungicides tested. Shrivastava and Verma (1989) worked on the fungicidal control of white rust of mustard in Sikkim. They compared the efficacy of nine fungicides against white rust of Brassica juncea coss (var. Varuna) at their scheduled dosage by spraying them at fortnightly intervals. Of these, Blitox-50 was found to be the most effective followed by Fytolan, Dithane Z-78 and Dithane M-45, both in controlling the disease as well as in increasing the yield. Singh and Singh (1990) tested nine fungicides in order to find out an economic fungicidal control of white rust disease. Out of all the fungicides Dithane M-45, 0.35 per cent reduced the disease in highest order as a result giving highest yield.
MATERIALS AND METHOD

Experiments conducted during the present investigation comprised laboratory, glass house and field studies.

The culture of *Albugo candida* used during the study was isolated from a diseased leaf of cv. Pusa bold (*Brassica juncea*) unless stated otherwise. Pusa bold plants were raised in 25 cm earthen pots at the rate of 5 in each. To obtain *A. candida* in pure culture, sporangial suspension from a single isolated mature pustule was made in 10 ml distilled water, maintaining a concentration of $10^5$ sporangia/ml (Verma, 1987) and uniformly sprayed on the leaves of 45 days old plants. Inoculated plants were incubated in a moist chamber for 24 hrs and then removed to the glass house for disease development. The pure culture of white rust, thus obtained, was further multiplied and maintained on Pusa bold plants to act as the source of inoculum for further experimental use unless stated otherwise.

Observations on rust intensity, whenever recorded was on the basis of per cent leaf area covered by rust pustules. The fifth leaf counted from the base of plants was considered for this purpose. Ratings for disease severity, which included both number, and size of pustules were expressed as 0, +, ++ and +++ to correspond to healthy, low, moderate and severe infection respectively.

For inducing floral malformation, young half opened flower buds of Pusa bold were inoculated with sporangial suspension of *A. candida*. After incubation in a moist chamber for 24 hrs, they
were removed to the glass house for the development of hypertrophy.

3.1 Identification of biological races of *Albugo Candida*.

A detailed study on the identification of biological races of *A. candida* occurring on various Brassica crops in India was carried out using a set of differential hosts.

The three infected *Brassica* spp. used to collect inocula for the experiment were *Brassica juncea* cv. Pusa bold, *B. nigra* and *B. campestris*, cv. PT 8824. The *B. juncea* and *B. nigra* collections were made from the IARI Farm, New Delhi while that of *B. campestris* was from Pantnagar area of Nainital district, Uttar Pradesh (India). After collection, they were maintained and multiplied on their respective host in the usual manner, to act as the source of inocula for cross inoculation purpose.

Seeds of the various Crucifer species for use as differential hosts, were obtained from the NBPGR, New Delhi; the Division of Genetics; the Division of Mycology and Plant Pathology, IARI, New Delhi; and the Crucifer Genetic Cooperative, University of Wisconsin - Madison, USA.

The cruciferous plants of both Canadian and Indian origin used as differential hosts for the present study were:-

1. *Raphanus sativus* cv. Pusa chetki (India)
   Differential host for Race 1

2. *Brassica juncea* cv. Pusa bold (India)
3. *Brassica juncea* cv. Varuna (India)
4. *Brassica juncea* cv. Domo (Canada)
   Differential hosts for Race 2
5. *Capsella bursa pastoris* (Canada)
   Differential hosts for Race 4

6. *Eruca sativa* (Taramira) (India)

7. *Sisymbrium officinale* (USA)
   Differential hosts for Race 5

8. *Rorippa islandica* (USA)
   Differential host for Race 6

9. *Brassica campestris* cv. Torch (Canada)
10. *Brassica campestris* cv. Tower (Canada)
11. *Brassica campestris* cv. Tobin (Canada)
12. *Brassica campestris* cv. PT 8824 (India)
13. *Brassica campestris* cv. T-9(Toria)(India)
   Differential hosts for Race 7

14. *Brassica nigra*
15. *Brassica napus* cv. NA-38
16. *Brassica napus* cv. NS-186
17. *Sinapis alba* (Canada)
   Differential hosts for Race 8.

Because of inavailability of seeds, *Armoracia rusticana* (horse radish), a differential host for *A. candida* race 3, could not be included in the list.
For inoculation, spores were collected from freshly ruptured pustules of infected field plants keeping samples from individual host plants separate. Spores were scraped from infected leaves, suspended in tap water and used for inoculation as described earlier.

The plants to be inoculated were grown in the manner stated earlier except in case of *Sisymbrium officinale*, *Capsella bursa pastòri* and *Rorippa islandica* where 15 to 20 plants were grown per pot.

After 15 days of inoculation, the infection severity on the most heavily infected plant, the per cent infected plants per pot and per cent diseased leaves per infected plant were recorded. Severity rating was based on both number and size of pustules. Ratings for disease severity were expressed as 0, +, ++, +++ which corresponded to healthy, low, moderate and severe infection respectively.

3.2 Establishment of dual culture of obligate parasite and its host using tissue culture techniques

3.2.1 Dual culture of *A. candida* in callus tissue of *B. juncea* (cv. Pusa bold)

*A. candida*, being an obligate parasite, cannot be cultured and maintained on artificial media. Tissue culture technique was, therefore, used to establish the culture of *A. candida* (the fungus) in the callus tissue of cv.Pusa bold (the host).

The experiment was carried out using materials and methods as elaborated further.
Source of explant

Plants of cv. Pusa bold were raised, inoculated with a sporangial suspension of a *B. junceae* culture of *A. candida* and incubated for disease development according to procedure described earlier.

Infected leaf bits collected from these plants after 15-20 days following incubation were used as explants in tissue culture experiments, for the establishment of dual culture whenever required. Explants obtained from uninoculated healthy plants of same age and vigour served as control.

To obtain hypertrophied floral organ as explant, emerging succulent inflorescence and young flower buds of potted plants were inoculated with white rust in the manner stated earlier and samples were collected after 45 days.

Besides these, naturally infected leaves and hypertrophied inflorescence of cv. Pusa bold collected from the field were also used as source of explants, whenever needed.

Sterilization of explants

All work relating to sterilization of explants, their transfer into tissue culture media tubes and/or flasks and subculturing of callus culture etc. were carried out under strict aseptic conditions in a transfer chamber having UV light and laminar flow facilities.

Instruments such as forceps, scalpel and needles etc. were
also sterilized by dipping them in 95 per cent ethanol followed by flaming and cooling. This was done at the start of the transfer work and several times during the operation.

Plant parts to be sterilized were washed with distilled water, treated with 0.1 per cent HgCl$_2$ solution for two minutes and then rinsed thrice with sterile distilled water to remove all traces of the sterilizing agent. Finally, they were blotted dry between folds of sterile blotting paper and transferred into media tubes.

**Preparation of media**

i. Composition

The basal medium used for *in vitro* studies of *Brassica* spp. was that of Murashige and Skoog (1962) supplemented with 0.8 per cent W/V agar and hormones as required. (Table 1 and 2).

ii. Preparation of stock solution of hormones

Stock solution was prepared separately for all the hormones needed to supplement the three different media used in the present investigation. These were prepared in acid clean volumetric flasks of either 50 ml or 100 ml capacity and stored at 4°C. Appropriate amount from these stock solutions was pipetted into the desired medium, whenever required. (Table 2).

(a). Stock solution of Indole butyric acid (IBA)

10 mg of IBA was dissolved in 1 to 2 ml of 1N NaOH and the volume was made upto 100 ml with distilled water. 4 ml of this
solution was used for every litre of MS medium for inducing callus formation.

(b). Stock solution of Benzyl amino purine (BAP)

50 mg of BAP was dissolved in 1-2 ml of 0.1N HCl. The volume was made upto 50 ml with distilled water, 2 ml of this stock solution was used for every litre of MS medium for inducing callus formation while 0.8 ml was used for regeneration (i.e., shoot formation).

(c). Stock solution of Napthyl acetic acid (NAA)

10 mg of NAA was dissolved in 1-2 ml of Absolute alcohol. The volume was made upto 100 ml with distilled water, 2 ml of this stock solution was used for every litre of MS medium to induce rooting.

(d) Stock solution of Indole acetie acid (IAA)

50 mg of IAA was dissolved in 1-2 ml of Absolute alcohol. The volume was made upto 50 ml with distilled water, 1 ml of this stock solution was used for every litre of MS medium for root formation.

(e) Stock solution of Kinetin (Furfuryl amino purine)

10 mg of Kinetin was dissolved in 1-2 ml 0.1 HCl and then the volume made upto 100 ml. 2 ml of this stock solution was used for every litre of MS medium for inducing rooting.
Table 1. Composition of Murashige and Skoog medium (1962)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (mg/l)</th>
<th>Moles (m mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major Inorganic Salts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{NH}_4\text{NO}_3 )</td>
<td>1650.00</td>
<td>20.60</td>
</tr>
<tr>
<td>K( \text{NO}_3 )</td>
<td>1900.00</td>
<td>18.80</td>
</tr>
<tr>
<td>Ca( \text{Cl}_2 \cdot 2\text{H}_2\text{O} )</td>
<td>440.00</td>
<td>2.99</td>
</tr>
<tr>
<td>Mg( \text{SO}_4 \cdot 7\text{H}_2\text{O} )</td>
<td>370.00</td>
<td>1.50</td>
</tr>
<tr>
<td>K( \text{H}_2\text{PO}_4 )</td>
<td>170.00</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>Trace Elements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_3\text{BO}_3 )</td>
<td>6.20</td>
<td>100.00</td>
</tr>
<tr>
<td>Mn( \text{SO}_4 \cdot 4\text{H}_2\text{O} )</td>
<td>22.30</td>
<td>100.00</td>
</tr>
<tr>
<td>Zn( \text{SO}_4 \cdot 7\text{H}_2\text{O} )</td>
<td>8.60</td>
<td>29.90</td>
</tr>
<tr>
<td>K( \text{I} )</td>
<td>0.83</td>
<td>5.00</td>
</tr>
<tr>
<td>Na( \text{2MoO}_4 \cdot 2\text{H}_2\text{O} )</td>
<td>0.25</td>
<td>1.03</td>
</tr>
<tr>
<td>Cu( \text{SO}_4 \cdot 5\text{H}_2\text{O} )</td>
<td>0.025</td>
<td>0.10</td>
</tr>
<tr>
<td>Co( \text{Cl}_2 \cdot 6\text{H}_2\text{O} )</td>
<td>0.025</td>
<td>0.105</td>
</tr>
<tr>
<td><strong>Iron Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe( \text{SO}_4 \cdot 7\text{H}_2\text{O} )</td>
<td>27.80</td>
<td>100.00</td>
</tr>
<tr>
<td>Na( \text{2EDTA} \cdot 2\text{H}_2\text{O} )</td>
<td>37.30</td>
<td>100.00</td>
</tr>
<tr>
<td><strong>Organic Supplement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meso Inositol</td>
<td>100.00</td>
<td>555.00</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.50</td>
<td>4.06</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>0.50</td>
<td>2.43</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>0.10</td>
<td>0.296</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.00</td>
<td>28.60</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30.00</td>
<td>87.60</td>
</tr>
</tbody>
</table>
Table 2. Amount of various hormones required to supplement MS medium (mg/l) to induce callusing, regeneration (shooting) and rooting.

<table>
<thead>
<tr>
<th>Nature of Medium</th>
<th>Kinetin</th>
<th>BAP</th>
<th>IBA</th>
<th>NAA</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callusing medium</td>
<td>-</td>
<td>2.0</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Regenerating medium</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rooting medium</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>1</td>
</tr>
</tbody>
</table>

iii. Procedure

In all experiments, Murashige and Skoog (MS) medium pack, supplied by M/s Hi-Media Laboratory, Bombay, India, containing sucrose, different vitamins and mineral salts was used after supplementing it with calcium, agar and hormones. CaCl₂ and Agar (Bacteriological grade) were supplied by M/s Glaxo laboratories, Bombay, India. All the hormones used i.e., BAP, IBA, NAA and IAA were obtained from Sigma Laboratories.

The sequences of steps involved in the preparation of the medium was as follows:

(a) 34.24 gms of MS basal medium (one pack) was dissolved in 750 ml of distilled water.

(b) It was supplemented with 332.24 mg of CaCl₂ and the required quantity of hormone(s) as indicated earlier. The volume of
the medium was then made up to 1000 ml with distilled water.

(c) The pH of the medium was adjusted to 5.8 using 0.1N NaOH and 0.1N HCl.

(d) Agar (0.8% W/V) was then added to the medium and heated till boiling.

(e) The molten medium obtained, was then dispensed into test tubes as well as in 50 ml and 100 ml conical flasks at the rate of 15, 15 and 25 ml respectively.

These were plugged with non-absorbent cotton, wrapped with a single layer of muslin cloth and then autoclaved at 121°C under 15 psi for 15 mts. After autoclavings, they were stored at room temperature till further use.

Culture ambience

All cultures were maintained at 18 ± 2°C under low intensity white fluorescent lamps providing an irradiance of 70 Umol M⁻² s⁻¹.

Histopathological studies

Microtome sections of healthy as well as A. candida infected callus tissue were obtained to study the fungal structure and their location according to the method of Johansen (1940). For these studies, 15 days old vigorously growing callus was used.

(a) Fixation

The materials were fixed in FAA fixing solution, the
composition of which was as follows:

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% Alcohol</td>
<td>90 ml</td>
</tr>
<tr>
<td>40% Formaldehyde</td>
<td>5 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

After 24 hrs, the material was transferred to 70 per cent alcohol.

(b) Dehydration and clearing

The fixed material was taken in passing tubes (rimmed tubes open at both ends with one end closed tightly with muslin cloth) and passed through the following alcohol and xylene series in the ascending order of their concentration respectively for dehydration and clearing.

Dehydration series -

- 70 per cent alcohol - 6 hrs; 80 per cent alcohol - 3 hrs;
- 90 per cent alcohol - 3 hrs; 95 per cent alcohol - 6 hrs;
- Absolute alcohol I - 24 hrs; and Absolute alcohol II - 24 hrs.

Clearing series -

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 : 1</td>
<td>Absolute alcohol : Xylene</td>
<td>3 hrs</td>
</tr>
<tr>
<td>1 : 1</td>
<td>Absolute alcohol : Xylene</td>
<td>3 hrs</td>
</tr>
<tr>
<td>1 : 3</td>
<td>Absolute alcohol : Xylene</td>
<td>3 hrs</td>
</tr>
<tr>
<td></td>
<td>Absolute xylene I</td>
<td>12 hrs</td>
</tr>
<tr>
<td></td>
<td>Absolute xylene II</td>
<td>12 hrs</td>
</tr>
</tbody>
</table>

(c) Infiltration, and embedding in Paraffin wax

After dehydration and clearing, the materials were transferred into specimen tubes each containing 5 ml Xylene. To this, a little
molten wax (E Merck's paraffin wax, MP - 55°-60°C) was added and kept at room temperature for 24 hours. The tubes were then placed in an oven at 60°C. At 4-hour intervals, a little of the homogenized solution was poured off and replaced with equal volume of molten pure paraffin wax. The process was repeated 4-5 times till there was absolutely no trace of Xylene in the tubes. On removal from the oven, the tissues were placed (in their proper orientation) in molten pure paraffin wax contained in coplin jar lids used as moulds. The paraffin wax was allowed to cool and the moulds, once formed, were neatly cut into small pieces, each containing the embedded host tissue. Each of these wax pieces was then mounted on a wooden block for sectioning.

(d) Sectioning

The wooden block was fixed to a rotary microtome and 13 μ thick ribbons containing sections of the embedded host piece were cut. These were carefully mounted on microslides using gelatin as adhesive. The composition of the gelatin adhesive used was Gelatin - 1 g dissolved at 35°C; Water - 90 ml; and Formalin - 10 ml.

The slides were then gently heated on copper bench to flatten the wax ribbons completely as well as to stretch the sections uniformly. The slides were then allowed to dry out completely for a day.

(e) Preparation of sections for staining

The slides containing the embedded sections were passed through the decending alcohol series to hydrate the sections.
Downgrade series -

Xylene I - 15 mts; Xylene II - 10 mts; Absolute alcohol - 15 mts; 95 per cent alcohol - 5 mts; 90 per cent alcohol - 5 mts; 80 per cent alcohol - 5 mts; 70 per cent alcohol - 5 mts; 50 per cent alcohol - 5 mts; 30 per cent alcohol - 5 mts; and Distilled water - 2 mts.

(f) Staining

Staining was done using cotton blue to stain fungal structures, and also with fast green in order to examine the callus tissue cells.

When staining with cotton blue, the sections were only passed down the series till distilled water and then stained with lactophenol-cotton blue. They were then covered carefully with a coverslip.

For staining with fast green, the sections were passed down the alcohol series and then also the upgrade series.

Upgrade series -

30 per cent alcohol - 5 mts; 50 per cent alcohol - 5 mts; 70 per cent alcohol - 5 mts; 80 per cent alcohol - 5 mts; 90 per cent alcohol - 5 mts; 95 per cent alcohol - 5 mts; Absolute alcohol I - 5 mts; Absolute alcohol II - 10 mts; 3 : 1 :: Absolute alcohol : Xylene - 10 mts; 1 : 1 :: Absolute alcohol : Xylene - 10 mts; 1 : 3 :: Absolute alcohol : Xylene - 10 mts; Absolute Xylene I - 5 mts; and Absolute Xylene II - 10 mts.

Sections were stained with fast green (in clove oil), mounted
in DPX mountant and covered with a coverslip. These slides were placed in an oven adjusted to 50°C for 24 hrs and then examined under the microscope. Photomicrographs were taken at various magnifications of the microscope.

3.2.2 Dual culture of *Peronospora parasitica* on callus tissue of *B. juncea* cv. Pusa bold

*P. parasitica* often occurs on Brassicas either alone or in association with *A. candida* and like the latter, is an obligate parasite which cannot be maintained on artificial medium.

For establishing dual culture of this obligate parasite and its host, tissue culture technique similar to that described earlier was used. The explants were obtained from Pusa bold plants pre-inoculated with a sporangial suspension of *P. parasitica* and also from white rust infected Pusa bold plants inoculated with *P. parasitica*. The inoculated plants were incubated for disease development as stated earlier. The leaves and inflorescence of such plants were used as source of explants during the study.

The other materials and methods used are similar to those described for dual culture of *A. candida* and its host.

3.3 Biochemical analysis

All biochemical analysis were carried out with two cultivars of *B. juncea*, Pusa bold (highly susceptible) and Bio-YSR (a yellow seeded regenerant - resistant). Seeds of cv. Bio YSR were obtained from the Biotechnology Division, N.R.L., IARI, New Delhi.
Pusa bold plants were raised in 25 cm earthen pots filled with well manured garden soil in the glass house at the rate of 5 plants in each. When 60 days old, they were uniformly inoculated with sporangial suspension of *A. candida* in the manner stated earlier. Plants sprayed with water served as control. Diseased leaf samples of approximately uniform size and from the same position of these plants, were harvested after 20 days following inoculation. Leaves of similar kind were also collected from the cv. Bio YSR plants of corresponding age groups.

To induce floral malformation, young inflorescence of 60 days old cv. Pusa bold were inoculated with sporangial suspension of *A. candida* in the manner stated earlier. Three stages in the development of staghead from cv. Pusa bold (hypertrophied inflorescence) i.e., (a) initial stage – tissue swelling incipient, (b) middle stage – tissue swelling pronounced, and (c) completely malformed stage – distortion and malformation complete, were chosen for analysis.

Healthy and diseased tissues were analysed for the estimation of total phenols, reducing and non-reducing sugars, free amino acids and crude protein and also macro and micro nutrients.

**Preparation of alcoholic extract**

Alcoholic extract of diseased and healthy tissues was prepared according to AOAC (1965). Healthy and diseased plant parts were separately collected, washed with double distilled water and then dried between folds of blotting paper. A 50 g sample of the desired material
was chopped into fine pieces, out of which exactly 5 g material was immediately plunged into 20 ml boiling ethanol (96%) in a beaker on a water bath. After boiling for 10 mts. the material was cooled on ice cold water, macerated and then the extract was centrifuged at 4000 rpm for 15 mts. The supernatant was filtered through Whatman No.42 filter paper and the volume was made upto 25 ml. This alcoholic extract was used for the estimation of total phenols, reducing and non-reducing sugars and free amino acids. Biochemical analysis for each metabolite was carried out in triplicate.

3.3.1 Estimation of sugars

Reducing and non-reducing sugars were estimated from the clarified alcoholic extract, by colorimetric, dinitrosalicylic acid (DNS) method (Miller, 1972).

Clarification

The clarification of alcoholic extract for the estimation of sugars was done according to AOAC (1965). Ten ml of alcoholic extract was heated in a boiler till all the alcohol was evaporated. To this 5 ml of 20 per cent ethanol was added followed by one ml saturated basic lead acetate solution to precipitate the colloidal substances present. The precipitate was removed by centrifugation at 6,000 rpm for 20 minutes. The excess of lead acetate in the supernatant was precipitated as lead oxalate by adding three ml of saturated potassium oxalate. It was filtered and the volume of the filtrate was made up to 10 ml with double-distilled water.
Reducing sugars

Reagents

i. Dinitro salicylic acid

100 ml of 1 per cent NaOH was taken in a beaker and to this 1 g DNS, 200 mg phenol and 50 mg sodium sulphite were added. The mixture was stirred well and stored in a bottle at 4°C.

ii. 40% solution of Rochelle - salt (Sodium Potassium tartarate)

Procedure

To 1 ml of clarified alcoholic extract, 1 ml of DNS reagent was added. The mixture was treated on a boiling water bath for 5 mts., and while the contents were still warm, 1 ml of 40 per cent Rochelle salt was added. The contents were mixed till effervescence ceased and then the volume was made up to 25 ml in a volumetric flask. The intensity of colour developed was measured at 575 nm in spectronic-20. The reducing sugar content was determined by referring to the optical density obtained by calibrated standard curve prepared from glucose.

Non-reducing sugars

Hydrolysis of clarified alcoholic extract of plant materials for the estimation of non-reducing sugars was carried out according to the method given by Malhotra and Sarkar (1979). One ml of clarified extract together with one ml of 1 N H₂SO₄ were taken in a test tube. The mixture was heated on a boiling water bath for half an hour and then the excess of H₂SO₄ was neutralised with 0.5 N NaOH solution using a drop of methyl-red as indicator. The solution was now made slightly acidic by adding a drop of acetic acid and the volume was made
up to five ml with distilled water. An aliquot of one ml from this solution was taken in a test tube and treated in the same manner as for reducing sugars to get the quantities of total reducing sugars. After substracting the value for reducing sugar from the total reducing sugars and multiplying it by the factor 0.93, the amount of non-reducing sugar was obtained.

3.3.2 Estimation of total phenols

The total phenol content was estimated according to the method of Swain and Hills (1959) as modified by Bhatia et al. (1972) using Folin Ciocalteu reagent. Estimation of phenols with this reagent was based on the reaction between phenols and an oxidising agent, phosphomolybdate which resulted in the formation of a blue complex (Bray and Thrope, 1954). The intensity of the coloured complex was measured colorimetrically.

Reagents

i. Folins Ciocalteu reagent

Commercially available reagent was diluted with equal amount of distilled water.

ii. 20% Na₂CO₃ solution

Procedure

One ml alcoholic extract of the required sample was taken in a test tube along with 0.5 ml Folin-Ciocalteu reagent and 2 ml of 20 per cent Na₂CO₃ solution. The content was thoroughly shaken, heated for one minute on a boiling water bath, and then cooled under running
water for colour development. The volume was made upto 25 ml with double distilled water and after half an hour, the optical density of the blue coloured solution was measured at 650 nm against a reagent blank.

The total phenol content in each sample was determined in reference to a standard curve prepared from graded concentrations of catechol.

3.3.3 Estimation of free amino acids

The total free amino acids in the extract was determined in terms of glycine-equivalent by the colorimetric method of Jacobs (1956). Amino acid groups react with ninhydrin (triketohydrindenehydrate) to give a coloured derivative, diketohydrindylidene-diketohydrindamine. This derivative has an absorbance maximum at 570 nm and can be conveniently measured colorimetrically.

Reagents
i) 0.2 M citric acid buffer (pH = 5.0)

21 g of citric acid was dissolved in 200 ml of 1N NaOH in a 500 ml volumetric flask and the volume was raised upto 500 ml with distilled water.

ii) Ninhydrin reagent

Solution of 800 mg of hydrated stannous chloride in 500 ml of the citrate buffer (pH 5.0) and 20 g of recrystalized ninhydrin in 500 ml of methyl-cellosolve were prepared separately and then mixed together. The reagent was freshly prepared before use.
iii) Diluent solution

It was prepared by mixing equal volumes of distilled water and n-propanol.

iv) Methyl red indicator

v) 0.1 N NaOH solution

Procedure

One ml of alcoholic extract was taken in a test tube together with a drop of methyl red indicator and then the solution was neutralized with 0.1 N NaOH. To this 1 ml of ninhydrin reagent was added and the mixture was heated on a water bath for 30 mts. It was cooled and the volume was made up to 10 ml with diluent solution and stirred. The optical density of the solution was measured at 570 nm.

The concentration of free amino acid in the solution was calculated from a standard curve prepared from different concentrations of glycine according to the procedure stated above. The total free amino acid concentration was expressed as mg glycine/g fresh weight of the sample material.

3.3.4 Estimation of mineral content

Minerals such as K were estimated by Flame photometer, while P was estimated by King's modification (1932) of Fiske-Subbarao's method. Atomic Absorption Spectrophotometer was used for the estimation of Cu, Fe, Mn, Zn, Pb, Cd and Co.

A 20 g sample material was cut and dried in the oven at 60°C. The sample was periodically removed, cooled in a dessicator
and weighed, and the process repeated until constant weight was obtained. The per cent moisture content of the sample was calculated by deduction of the final dry weight from its fresh weight.

Processing of the samples for the determination of mineral content was done by wet digestion using acid mixture. For N, P and K analysis, 1 g of the experimental material was digested with diacid mixture of sulphuric and perchloric acid (9:1 v/v) and the volume was made up to 100 ml after its quantitative transfer through filtration into a 100 ml volumetric flask. For analysis of other nutrients i.e., Cu, Fe, Mn, Zn, Pb, Cd and Co, triacid mixture of nitric, sulphuric and perchloric acid (10:1:1 v/v) (Piper, 1966) was used instead of the diacid mixture.

Total nitrogen estimation

The total nitrogen content was determined as ammonia per gram material by the Kjeldahl method (Piper, 1966). Weighed sample (0.5 g) was taken in a digestion flask. To this a mixture of potassium sulfate (1.0 g), copper sulfate (0.3 g) and concentrated sulphuric acid (10 ml) was added. The contents of the flask were thoroughly mixed by shaking and then a few zinc granules were added in the flask to prevent bumping. Digestion was carried out till the mixture turned transparent green. Then the digested material was transferred into a round bottom flask by washing with 100 ml distilled water. To this 100 to 120 ml of 40 per cent sodium hydroxide was added to make the solution alkaline. Digested material was distilled, the evolved ammonia was trapped in 25 ml of 0.1 N sulphuric acid containing three drops of
methyl red indicator. Excess of sulphuric acid left was titrated back against 0.1 N sodium hydroxide. Volume of sulphuric acid used to trap ammonia was calculated.

Estimation of phosphorus

King's modification (1932) of Fiske Subbarao's method was used. The acid digested sample in the solution form was treated with molybdic acid, the phosphorus present is converted into phosphomolybdic acid which is then selectively reduced by 1, 2, 4 amino-naptholsulphonic acid reagent resulting in a blue colour. The intensity of the colour which is proportional to the amount of phosphate present was then read at 660 nm.

Reagents

i. Amino-napthol sulphonic reagent

195 ml of 15 per cent sodium bisulphite solution was taken in a glass stoppered cylinder and 0.5 g of 1, 2, 4 amino-naptholsulphonic acid was added to it followed by 5 ml of 20 per cent sodium sulphite. The solution obtained was transferred to a brown glass bottle and stored at low temperature. The reactive state of the solution lasts for 4 weeks.

ii. Ammonium molybdate solution (25%)

2.5 g of ammonium molybdate was dissolved in distilled water, transferred into a 100 ml volumetric flask and the volume was made up to the mark.

iii. Standard phosphate

Exactly 351 mg of pure dry monopotassium phosphate (KH₂PO₄)
was dissolved in distilled water and then transferred quantitatively into a volumetric flask. To it, 10 ml of H₂SO₄ (10 N) was added, mixed thoroughly and the volume was made up to the mark. Thus, the solution contained 0.4 mg of phosphorus in 5 ml.

Procedure

2 ml of the acid digested material was taken in a 25 ml volumetric flask, then 1.2 ml perchloric acid was added, followed by the addition of 1.0 ml of 2.5 per cent ammonium molybdate and 0.5 ml 1, 2, 4 aminonaphthol sulphonic acid. The blue complex obtained was read at 660 nm.

Estimation of potassium

The estimation was done with the help of Flame Photometer. The diacid digested sample solution was introduced in the form of a fine continuous spray into a non-luminous gas flame. The emitted light, characteristic for the ion being analysed was isolated and focussed on a photoelectric cell and the current intensity was measured on a suitable meter.

Procedure

A stock solution was prepared by dissolving 1 g equivalent of the mineral salt in glass distilled water so as to attain the concentration of 1000 μg/ml. Suitable aliquots were taken and diluted to obtain a standard curve within the range of determination. The standard and blank solutions of the mineral were aspirated into the flame directly or after suitable dilutions to attain working range of the instrument, thereafter, the samples were read.
Cu, Fe, Mn, Zn, Pb, Cd and Co estimation

The estimations were carried out using Perkin-Elmer 2380 Atomic Absorption Spectrophotometer.

A suitable aliquot was sprayed into the flame of the instrument and the absorption or emission of the mineral to be analysed was measured at a wavelength which was different for different minerals.

3.3.5 Estimation of crude protein

Approximate value for per cent soluble protein per milligram dry weight of the material was determined by multiplying the percentage nitrogen contained in the sample by the factor 6.25.

3.4 Chemical control

A field experiment was conducted during the 1988-89 and 1989-90 crop seasons with three systemic and three contact fungicides to evaluate their efficacy in controlling white rust and Alternaria Leaf Spot of rapeseed and mustard.

The following fungicides were used:

Systemic

1. Oxadixyl (0.2%w/w)  2 Methoxy-N-(2-oxo-1,3-oxazolidin-3yl)
2. Topsin (Cercobin) (0.2%w/w)  1,2 di (3 ethoxycarbonyl-2-thioureido)
3. Ridomil (metalaxyl) (0.15%w/w)  Methyl-DL-N-(2,6 dimethyl phenyl phenyl-N-(2-methoxyacetyl)-alaninate.
Non systemic

4. Chlorothalonil (Bravo, Daconil) (0.2% conc)
5. Rovral - (0.2% conc)
6. Dithane M-45 (mancozeb) (0.2% conc)

tetrachloroisophthalonitrile
3-(3,5-dichlorophenyl)-N-isopropyl-carbomoyl-2,4-dioxoimidazolidine-1-carboxamide
Zinc ion + manganese - ethylene bis dithiocarbamate.

The experiment was carried out at the IARI farm, New Delhi, in Randomised Block Design with three replications using the cultivar Pusa bold. Seeds were sown in 2 x 4 m plots in rows, maintaining a spacing of 30 cm between the rows and 10 cm between the plants during the 3rd week of October each season.

There were four spray schedules for each of the three systemic fungicides, all starting on the same date but each terminating on a definite date.

The first spray schedule consisting of only one spray for each of the six fungicides was applied on the 1st of January when plants were 65 days old.

The second schedule consisted of two sprays of these chemicals applied on 1st January and 15th January.

Similarly, the 3rd spray schedule starting on 1st January, terminated on 30th January and comprised of three sprays for each chemical while the fourth consisted of four spray treatments for each
fungicide applied at 15 days interval starting from 1st January and terminating on 14th February.

Plots sprayed with contact fungicides received all the above four sprays at 15 days interval starting from 1st January. Plots kept as control did not receive any fungicidal spray. Observations on disease intensity were recorded on the 10th day following the final spray.

For measuring disease intensity, one leaf from about 20 inches height from the base of a plant was harvested and all such leaves from 10 randomly selected plants of a treatment were considered to determine the average percentage of leaf area damaged by the disease.

The crop was harvested in the 1st week of April and seed yield of individual plots was recorded. The data was discussed at 5 percent level.
RESULTS

4.1 Identification of biological races of *A. candida*

Information on the prevalence of biological race(s) of *A. candida* attacking oeliferous Brassicas in India is lacking. Hence, the present experiment was taken up with three isolates of the pathogen parasitizing three commonly cultivated species of Brassica viz., *B. juncea* (mustard), *B. campestris* (yellow sarson) and *B. nigra* (black sarson). These isolates were tested on a set of differentials to ascertain their race identity in a glass house keeping proper isolation distance. Disease severity on the most heavily infected plant, the per cent infected plants per pot and per cent diseased leaves per plant were recorded after 15 days following inoculation. Ratings for disease severity were expressed as 0, +, ++ and +++ which corresponded to healthy, low, moderate and severe infection respectively.

i. *B. juncea* isolate

The results obtained are summarized in Table 3. Out of the three cultivars of *B. juncea* namely, Pusa bold, Varuna and Domo inoculated with the *B. juncea* isolate of *A. candida*, the first two showed highly susceptible (+++C) reaction to the disease. Both cotyledonary and mature leaves of these plants showed severe infection. The percentage of infected plants in case of cvs. Pusa bold and Varuna were respectively, 90.0 and 91.8 and that of infected leaves were respectively 48 and 50. Domo proved to be resistant.
Table 3. Reaction of differential host species to *B. juncea* isolate of *A. candida*.

<table>
<thead>
<tr>
<th>Differential hosts</th>
<th>Disease severity*</th>
<th>% Plant Infected</th>
<th>% Leaves Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Raphanus sativus</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. juncea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Pusa bold</td>
<td>+++ cl</td>
<td>90.0</td>
<td>48</td>
</tr>
<tr>
<td>cv. Varuna</td>
<td>+++ cl</td>
<td>91.8</td>
<td>50</td>
</tr>
<tr>
<td>cv. Domo</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Capsella bursa-pastoris</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Eruca sativa</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sisymbrium officinale</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rorippa islandica</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. campestris</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Torch</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tower</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tobin</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. PT 8824</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. PT 303</td>
<td>+++ cl</td>
<td>64.2</td>
<td>12</td>
</tr>
<tr>
<td>cv. T-9 (Toria)</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>+++ cl</td>
<td>100.00</td>
<td>50</td>
</tr>
<tr>
<td><em>B. napus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. NA-38</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. NS-186</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sinapis alba</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* 0 = healthy; + = low; ++ = Moderate; +++ = severe;  
C = cotyledonary leaves; cl = both cotyledonary and mature leaves.
B. nigra also showed high degree (++++) of susceptibility to this isolate of A. candida and all the inoculated plants got infected with the disease. Fifty per cent of leaves in these plants were infected.

In contrast, out of the six cultivars of B. campestris inoculated with this B. juncea isolate of A. candida only the cultivar PT-303 showed high degree (+++) of susceptibility. Although 64.2 per cent of plants of the above cultivar contacted the disease, only 12 per cent of their leaves showed the presence of rust pustules.

Further more, artificial inoculation of Raphanus sativus Capsella bursa pastoris, Eruca sativa, Syssymbrium officinale Rorippa islandica, B. napus and Sinapis alba plants with the above mentioned white rust isolate did not give positive result.

All these results show that B. juncea isolate of A. candida used in the present experiment is similar to race-2 of the pathogen since it is highly virulent on the cultivars of both B. juncea and B. nigra but not on those of B. campestris except the cultivar PT 303.

ii. B. campestris isolate

From the results of inoculation experiment presented in Table 4, it is apparent that the B. campestris isolate of A. candida showed only low (+) to moderate (++) disease intensity on the four cultivars of B. campestris out of the six inoculated. The cultivars Tobin, PT-8824, PT 303 and T-9 (Toria) respectively, 3.8, 13.33, 18.7 and 15.0 per cent infected plants. The percentage of infected leaves in these cases were 3, 5, 4 and 5 respectively. None of the remaining plants proved susceptible to the isolate, although B. campestris is the
Table 4. Reaction of differential hosts *species of* of \textit{A. candida} of \textit{B. campestris} isolate

<table>
<thead>
<tr>
<th>Differential hosts</th>
<th>Disease severity*</th>
<th>% Plant Infected</th>
<th>% Leaves Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Raphanus sativus}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{B. juncea}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Pusa bold</td>
<td>$^{+c_1}$</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>cv. Varuna</td>
<td>$^{++c_1}$</td>
<td>90.9</td>
<td>20</td>
</tr>
<tr>
<td>cv. Domo</td>
<td>$^{+c}$</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>\textit{Capsella bursa-pastoris}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Eruca sativa}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Sisymbrium officinale}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Rorippa islandica}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{B. campestris}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Torch</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tower</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tobin</td>
<td>$^1$</td>
<td>3.8</td>
<td>3.0</td>
</tr>
<tr>
<td>cv. PT 8824</td>
<td>$^1$</td>
<td>13.33</td>
<td>5.0</td>
</tr>
<tr>
<td>cv. PT 303</td>
<td>$^{++1}$</td>
<td>18.7</td>
<td>4.0</td>
</tr>
<tr>
<td>cv. T-9 (Toria)</td>
<td>$^{++c_1}$</td>
<td>15.0</td>
<td>5.0</td>
</tr>
<tr>
<td>\textit{B. nigra}</td>
<td>$^{++c_1}$</td>
<td>42.85</td>
<td>20</td>
</tr>
<tr>
<td>\textit{B. napus}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. NA-38</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. NS-186</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Sinapis alba}</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* 0 = healthy; $^+$ = low; $^{++}$ = moderate; $^{+++}$ = severe;
\(c\) = cotyledonary leaves; \(c_1\) = both cotyledonary and mature leaves.
original host for this pathogen.

On the other hand, all the three cultivars of B. juncea took up infection. In case of cvs. Pusa bold and Varuna, both their cotyledonary and mature leaves showed moderate \((++^{cl})\) disease intensity whereas Domo showed low \((+^{c})\) disease intensity with only few pustules on its cotyledonary leaves. The percentage of infected plants in case cvs. Pusa bold, Varuna and Domo was 90, 90.9 and 16 respectively. Whereas the percentage of infected leaves in the above three cases was 10, 20 and 4\textperthousand respectively.

Plants of B. nigra also showed moderate \((++^{cl})\) disease intensity both on their cotyledonary and mature leaves. The percentage of infected plants and infected leaves was 42.85 and 20 respectively.

No positive reaction was obtained when the inoculation experiment was carried out with Raphanus sativus, Capsella bursa-pastoris, Eruca sativa, S. officinale, Rorippa islandica, B. Napus (cvs. NA 38 and NS 186) and Sinapis alba.

Thus, it is evident that the B. campestris isolate of A. candidatus showed high degree of infection on both B. juncea and B. nigra cultivars like that showed by the B. juncea isolate of the pathogen but it differed from the later isolate in its pathogenicity on B. campestris, where it could induce low to moderate reaction, in only four cultivars out of the six inoculated.
iii. *B. nigra* isolate

From the results of inoculation tests presented in Table 5, it is evident that the *B. nigra* plants showed highest (+++ disease severity when they were subjected to inoculation with *B. nigra* isolate of *A. candida*. The percentage of infected plants and leaves were 100 and 30 respectively.

Similarly, out of the three *B. juncea* cultivars inoculated namely, Pusa bold, Varuna and Domo, the first two showed infection while Domo proved resistant to this isolate of *A. candida*. The cvs. Pusa bold and Varuna showed highest (+++\textsubscript{cl}) disease severity although cotyledonary stage of Varuna did not take up infection. Percentage of plants and leaves infected in case of cv. Pusa bold was respectively, 100 and 20 while in case of Varuna it was 40 and 16 respectively, for plants and leaves.

In case of *B. campestris*, however, out of the six cultivars inoculated with *B. nigra* isolate of white rust, only three cultivars viz., PT 8824 (+\textsuperscript{C}), PT 303 (++\textsuperscript{C}) and T-9 (Toría) (+\textsuperscript{C}) showed susceptible reaction at the cotyledonary stage. The percentage of plants infected in the three cases were 16.66, 33.3 and 10 respectively. The percentage of leaves infected in the three cases was respectively, 5, 6 and 2. The cultivars Torch, Tower and Tobin reacted resistant and did not take up infection.

Cultivars of *B. napus* also showed susceptible reaction to the white rust isolate from *B. nigra*. Both the cultivars namely, NA-38 and NS-186 showed infection at the cotyledonary leaf stage
Table 5. Reaction of differential host species to *B. nigra* isolate of *A. candida*.

<table>
<thead>
<tr>
<th>Differential hosts</th>
<th>Disease severity*</th>
<th>% Plant Infected</th>
<th>% Leaves Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Raphanus sativus</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. juncea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Pusa bold</td>
<td>+++cl</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>cv. Varuna</td>
<td>+++l</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>cv. Domo</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Capsella bursa-pastoris</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Eruca sativa</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sisymbrium officinale</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rorippa islandica</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. campestris</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. Torch</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tower</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. Tobin</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cv. PT 8824</td>
<td>+c</td>
<td>16.66</td>
<td>5</td>
</tr>
<tr>
<td>cv. PT 303</td>
<td>++c</td>
<td>33.3</td>
<td>6</td>
</tr>
<tr>
<td>cv. T-9 (Toria)</td>
<td>+c</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>+++cl</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td><em>B. napus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv NA-38</td>
<td>+c</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>cv. NS-186</td>
<td>+c</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><em>Sinapis alba</em></td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*0 = healthy; + = low; ++ = Moderate; +++ = severe; c = cotyledonary leaves; cl = both cotyledonary and mature leaves.*
although the disease severity was very low (+c). Similarly, the percentage of infected plants and infected leaves in the two cases was low, being respectively, 10 and 2 in case of NA-38 and 5 and 4 in case of NS 186.

*Raphanus sativus, Capsella bursa-pastoris, Eruca sativa, Sisymbrium officinale, Rorippa islandica* and *Sinapis alba* again did not take up infection.

Results of this experiment also show highest severity of the disease on the two *B. juncea* cvs. Pusa bold and Varuna besides *B. nigra*, which are similar to the results obtained in the previous two inoculation experiments.

It can thus be concluded that the three isolates of *A. candida* behaved more or less similarly although some differences were observed in the degree of severity on certain differential hosts.

4.2 Establishment of dual culture of obligate fungal parasite and its host using tissue culture techniques

Tissue culture offers convenient substrates for the growth of obligate parasites under controlled and sterile environmental conditions making it possible to maintain axenic culture and also making precise physiological investigations feasible.

In the present study, experiments were taken up to establish dual culture of the obligate parasites *A. candida* and *Peronospora parasitica* on their common host, *B. juncea* cv. Pusa bold.
Production of healthy Brassica callus and plantlet

Callus cultures as well as plantlets of cv. Pusa bold were produced on the callusing medium using either leaves, stem or inflorescence axis of the plant as explant. Their growth was slightly faster when leaf explants were used as compared to explants obtained from either stem or inflorescence axis.

Leaves after their transference into the callusing medium, responded by gradual change of their colour from dark to lighter green and thickening of their tissues. The first sign of callusing was discernable after 6 to 7 days when the explant turned yellowish white at its cut ends and small nodules of white glistening cells, and in less frequent cases, small rootlets, appeared from its cut edges. Proper callusing began after 10-12 days, the growth of which started first at the cut edges but eventually the entire explant was transformed into a callus mass. The callus continued to increase in size by putting forth fresh cells, the rate of growth of which decreased with age. After about 25 days of incubation at $18\pm2^\circ$C, no further growth was observed. This was followed by browning and senescence of the callus.

Subculturing of active callus tissue into fresh callusing medium resulted in cent per cent success in obtaining fresh callus growth but when done after the onset of browning, no regeneration took place. The optimum time interval required for subculturing of a callus for its regeneration was found to depend on its incubation temperature. The period of subculturing of the callus could be increased by lowering the temperature of its incubation (Table 6).
Table 6. Relationship between incubation temperature and interval of subculturing.

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>Interval of subculturing (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
</tr>
</tbody>
</table>

Stem explants when introduced into the callusing medium responded, like the leaf explants, by change of their colour to lighter green and exhibited proliferation and callusing at their cut edges which are in contact with the medium. When the stem explants were placed vertically into the medium, initiation of callusing took place at the end which was within the medium. Callus formation and time of subculturing were similar to those described in respect of leaf explants.

To obtain plantlets in vitro, the standard procedure is the transference of the callus, first into the regenerating or shooting medium followed by their subsequent transfer into the rooting medium after 2-3 weeks. But interestingly during the course of the present experiment, root and shoot formation were observed during growth of the callus in the callusing medium itself. Initiation of rooting was observed as early as 7 days after the explants were introduced into the callusing medium in 20 per cent of the cases. The rootlets grew up to a maximum of 2 cm in length, penetrating deeper into the agar medium and putting forth fine lateral root hairs (Plate 3). In some cases rootlets were
Plate 3. Explant showing root formation from the embedded region.

AND

Plate 4. Explant showing root formation from the exposed region.
Plate 5. Shoot formation from callus of cv. Pusa bold.

Plate 6. Profuse growth of shoots.
produced from the region of the explant not embedded in the medium (Plate 4).

Shoot formation was, however, observed on 15 to 20 day old callus. Such callus exhibited darker greenish areas which readily produced shoots when subcultured into the callusing medium (Plates 5 and 6). Shoot bud differentiation took place from peripheral cells. Sometimes shoots were produced even when no subculturing was done. Plantlets could be produced without the formation of rootlets. In such a case, the callus acted as the absorptive organ. As the plantlet grew no increase in size of the callus was observed but slight browning of the latter took place.

The plantlets survived if subcultured at intervals of 20-25 days at the incubation temperature of $18 \pm 2^\circ C$. If subculturing was delayed, the plantlets showed gradual yellowing after 30 days.

4.2.1 Dual culture of *A. candida* in callus tissue of cv. Pusa bold (*B. juncea*)

The various methods tried for the establishment of dual culture of *A. candida* and its host are as follows:

i. **Inoculation of healthy host callus tissue with *A. candida* sporangia**

Host: Callus culture of cv. Pusa bold was established on callusing medium in test tubes according to the method stated earlier. Fully formed vigorously growing, 15 days old callus was chosen for the experiment.
Parasite: Leaves from 25 days old plant of cv. Pusa bold bearing widely dispersed white rust pustules were selected for the purpose. Leaf pieces, each containing a mature but unruptured pustule as well as those having a mature but freshly ruptured pustule, were cut out under a binocular microscope to serve as the source of inoculum.

Establishment of dual culture: The leaf explants were surface sterilized and sporangial masses from their rust pustule were used to inoculate a healthy host callus under aseptic condition. Sporangia from only one pustule was used to inoculate a healthy callus. A droplet of sterile water was introduced into the test tube to moisten the callus surface. After plugging, the tubes were incubated at $18\pm2^\circ C$.

Inoculation of healthy callus of cv. Pusa bold with sporangia obtained from ruptured white rust pustule yielded only various fungi as secondary invaders (contaminants) but not *A. candida*. Growth of these organisms not only covered the callus tissue but they also ramified on to the surrounding medium in all cases.

However, when sporangial inoculum was taken from an unruptured pustule to inoculate the callus, no visible change in the callus could be noticed. Microscopic examination of sections of callus tissue taken after 7, 14 and 21 days of inoculation with such sporangial material also did not reveal the presence of any fungal mycelium.

ii. Use of *A. candida* infected leaf pieces as explants

Cotyledonary leaves of cv. Pusa bold were washed with sterile water and then inoculated by spraying sporangial suspension
(concentration $10^5$ sporangia/ml) of $A. candida$ in sterile water, with the help of an atomizer. These were placed in moist chambers made of transparent plastic petri plates lined with sterile moistened filter paper and incubated at $18\pm2^\circ C$.

The infected leaves were surface sterilized after 48 hrs., each of them was cut into two parts and then transferred aseptically into callusing medium tubes at the rate of one in each, in such a way that they remained half embedded into the medium. Some of these explants, grew into normal callus tissues. However, microscopic examination of hand sections of these callus tissue after 7, 14 and 21 days also did not reveal the presence of any fungal structure.

A few explants showed slight thickening of their tissues without any callus formation. There was, however, no further change in their conditions upto two weeks from the time of their transfer into the medium, after which they turned yellow followed by browning and death.

iii. Use of leaf explants from the margin of white rust pustule

Leaf bits of approximately 0.1 x 0.7 cm size cut from the margin of unruptured white rust pustules, were surface sterilized in the usual manner and then aseptically introduced into the callusing medium tubes at the rate of one in each. The tubes were kept under incubation in the manner stated earlier.

It was observed that about half of these explants did not have any growth and their margins turned brown within four days of introduction
into the medium. From their cut margins, cottony growth of sterile hyphae emerged which eventually covered them leading to death of the explant.

In 33.38 per cent of the cases, the explants retained their green colour for a few days but later turned yellow and died. Another 16.66 per cent of the explants, however, showed limited callusing from their cut edges, small blister like bodies appeared from their exposed surface on the 10th day which soon developed into rust pustules (Plate 7). These gradually matured and eventually released the spores onto the medium, 16 days after the explants were introduced into the medium. The portion of the explant bearing the pustule eventually became necrotic.

iv. Use of leaf explants having a single white rust pustule

Leaves of cv. Pusa bold having unruptured as well as freshly ruptured white rust pustules were selected for the present study. These were aseptically cut into small pieces of 1.0 cm x 0.7 cm in such a way that each piece contains a single pustule. Each of these bits were then introduced into callusing medium tubes at the rate of one in each and then kept for incubation.

The response of explants having ruptured pustule placed either on the surface or partly embedded into the medium, as well as those with unruptured pustule similarly placed, were different (Table 7).

Explants having ruptured pustule when placed on the medium
Plate 7. *In vitro* formation of white rust pustule on explant surface.
Table 7. Response of leaf explants with white rust pustule on the callusing medium.

<table>
<thead>
<tr>
<th>Nature</th>
<th>Position on the medium</th>
<th>Per cent yielding sterile fungus</th>
<th>Per cent supporting growth and and rupture of pustule</th>
<th>Per cent explants yielding <em>P. parasitica</em></th>
<th>Per cent contaminated</th>
<th>Per cent showing no response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf with ruptured pustule</td>
<td>Surface of the medium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Embedded in the medium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Leaf with unruptured pustule</td>
<td>Surface of the medium</td>
<td>46.66</td>
<td>6.66</td>
<td>13.33</td>
<td>13.33</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Embedded in the medium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>
either on or partly below the surface, invariably yielded fungi and bacteria suggesting thereby that these are the secondary invaders.

When explants bearing unruptured pustule were placed on the medium surface, 46.66 per cent of them showed thickening within the first six days and accompanied by a change in colour from dark green to lighter green. Cottony growth of sterile hyphae was also seen emerging from their cut edges and on the 10th day, it spread from the explant onto the surrounding medium. This was followed by gradual death of the explant due to overgrowth of the sterile fungus.

However in 6.66 per cent cases, maturation of the young unruptured pustule and partial callusing of the explant were observed. Within six days of introduction of the explant into the medium, the young pustule matured, the explant remained fresh but gradually turned into lighter green colour. On the 8th day, the pustule ruptured in vitro (Plates 8 and 9), releasing the spores and by the 13th day, the portion of the explant bearing the pustule turned yellow, shrivelled and eventually dried up (Plate 10). The lower region of the explant within the agar but devoid of pustule showed normal callus growth. However, microscopic examination of the callus tissue formed did not reveal the presence of any fungal body within it. The callus exhibited a very slow growth which ceased after 20 days.

In 26.66 per cent cases during the 1st week of their transfer, explants showed thickening accompanied by a change in their colour to lighter green with yellowing at their margins but no callusing occurred. At the end of a week, a fungal growth was observed around the pustule
Plate 8. *In vitro* maturation of white rust pustule on cv. *Pusa* bold leaf explant.

AND

Plate 10. Explant showing necrosis at the region of white rust pustule.
present on the explant. On microscopic examination, the fungal growth was identified as that of \textit{Peronospora parasitica}. In half of the above cases (13.33%), pure culture of \textit{P. parasitica} was only obtained, while in the remaining cases (13.33%), the growth of \textit{P. parasitica} together with a sterile fungus of unknown identity was observed.

In cases where the explant yielded the pure growth of \textit{P. parasitica} the host tissue around the rust pustule showed further yellowing although the downy mildew pathogen showed dense growth and sporulation. However, within 20 days yellowing following by browning and ultimate wilting of the explant took place accompanied by cessation of growth of the pathogen. Details regarding the growth of the downy mildew fungus has been described under the dual culture studies of \textit{P. parasitica} and its host.

In case of mixed infection of the explant with \textit{P. parasitica}, and the sterile fungus, the growth of the latter was faster, within a week it completely covered the explant and ramified onto the surrounding medium. Their combined effect being the rapid death of the explant. Twenty per cent of the explants, however, showed growth of saprophytic fungi and bacteria.

In contrast, when explants with unruptured pustule were placed on the medium, they showed no growth response.

v. Use of different parts of hypertrophied inflorescence of \textit{B. juncea} cv. Pusa bold as explants

Success in the establishment of dual culture of \textit{A. candida} and its host, depended upon the selection of appropriate stage of the
hypertrophied inflorescence.

Mature purple coloured stagheads with fibrous tissue proved unsuitable for use as explant. They exhibited only limited or no growth in the callusing medium. Also, if stagheads having the growth of P. parasitica on their surface are used as explants, high percentage of contamination is obtained.

Pieces of green stagheads showing prominent thickening but free from P. parasitica infection proved to be most suitable explant which gave consistently good result. Malformed inflorescence (staghead) was obtained after artificially inoculating young flower buds of cv. Pusa bold with a single pustule culture of B. juncea pathotype of A. candida according to the manner stated in the earlier chapter (Ref. 3.2.1).

After surface sterilization of the staghead, its different parts were separated and aseptically transferred into the callusing medium tubes to obtain the dual culture. Healthy counterparts were kept as control wherever possible. These explants differed widely in their growth response in the callusing medium (Table 8 and Fig.1.) as explained below:

A) Thickened flower bud: When thickened green flower buds from a hypertrophied inflorescence were placed into the medium, they showed limited enlargement and callusing occurred at cut ends only. Callusing was rated as poor (+).

B) Sepals: Such explants exhibited no change either in size or in shape in the callusing medium but their colour changed to yellow. No further change was noticed in the explant with time.
Table 8. Percentage of various types of systemically infected explants producing callus growth in callusing media.

<table>
<thead>
<tr>
<th>EXPLANTS</th>
<th>Flower bud</th>
<th>Sepal</th>
<th>Petal</th>
<th>Anther</th>
<th>Ovary</th>
<th>Leaf</th>
<th>Leaf with unruptured pustule</th>
<th>Inflorescence axis</th>
<th>Inflorescence axis with unruptured pustule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.33%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>66.66%</td>
<td>66.66%</td>
<td>100%</td>
<td>50%</td>
</tr>
</tbody>
</table>
CALLUSING RESPONSE

0 NONE  + POOR  ++ LOW  +++ GOOD  ++++ EXCELLENT

FIG. 1: CALLUSING ABILITY OF DIFFERENT PARTS OF *Albugo candida*

INDUCED HYPERTROPHIED INFLORESCENCE
C) Petals: These explants, after 3 days of introduction into the medium, showed slight browning and 70 per cent of them died within 10 days. In the remaining 30 per cent of the cases, limited enlargement and callus formation took place initially which ceased by the 15th day. Similar response was exhibited by those kept as control.

D) Anthers: When anthers were used as explant, no positive response towards callus formation was observed. They gradually turned brown and died. Healthy anthers used as controls behaved in the similar way.

E) Ovary: The ovary (excised from the hypertrophied inflorescence), when placed into the callusing medium showed slight thickening during the first 5 days. Limited callus formation also took place from its cut end but no further growth was observed even upto the 20th day. Healthy ovary kept as control exhibited slight thickening only.

F) Thickened apical leaf: When such explants were placed into medium, they showed enlargement within the first 3-4 days and by another 7 days, prominent light greenish white callus formed at the cut ends. The callus development was rated as good (+++). By the 20th day, more than two third portion of the explant was converted into callus mass after which callusing activity slowed down. After 29th day following introduction of the explant into the medium, it started browning.

G) Thickened apical leaf bearing unruptured pustule: In case of thickened apical leaf pieces bearing young white rust pustule, enlargement and callusing was similar to those without rust pustule but callus development was comparatively less prolific and was rated as low (++).
Maturation and rupturing of the pustule took place after the 3\textsuperscript{rd} day. The percentage of such explants that survived were 66.6 per cent as compared to 85 per cent in case of the former.

H) Thickened inflorescence axis : When 0.5 inch long pieces of thickened inflorescence axis were transferred into the medium, they responded by showing further thickening and change of colour to light green. Prominent signs of callusing were evident at the two cut ends within the first 7 days (Plate 11). Rapid callusing progressed from the cut margins towards the centre of the explant and within 10 days of its transfer into the callusing medium, the entire body of the explant was converted into a mass of callus tissue (Plate 12). The callusing was rated as excellent (++++). On the other hand its healthy counterpart showed callusing only at the cut edges, callusing being more at the end immersed into the medium (Plate 13).

The infected callus formed was white in colour with glistening actively dividing cells on the surface. The callus showed vigorous growth, covering the entire surface of the medium within 20 days of its introduction into the medium, after which the growth slowed down. If retained in the same medium beyond 25 days without subculturing, the callus turned flaccid and brown in colour.

The callus derived from such explants never gave rise to plantlets or formed roots as is generally observed in case of callus derived from healthy explants of cv. Pusa bold.

I) Thickened inflorescence axis with unruptured rust pustule : Explants
Plate 11. Explant from hypertrophied inflorescence axis after a week's growth in callusing medium.
Plate 12. *A. candida* infected explant (inflorescence axis) converted into a callus after 10 days' growth in callusing medium.

Plate 13. Healthy explant (inflorescence axis) showing callusing at cut ends after 10 days' growth in callusing medium.
of this type showed response similar to thickened inflorescence axis described above. Callusing was, however, restricted to the region devoid of pustule and could be rated as very good (+++).

The nature of response of different parts of *A. candida* induced hypertrophied inflorescence when used as explant in the callusing medium after 5, 15 and 25 days of incubation is summarised in Table 9.

vi. **Inoculation of *in vitro* grown plantlet with**

*A. candida* sporangia

A plantlet of cv. Pusa bold was grown *in vitro* according to the procedure elaborated earlier. Maintaining aseptic conditions the plantlet was inoculated with spores from an unruptured pustule of white rust. A droplet of sterile water was introduced into the test tube to moisten the surface of inoculated leaf. It was then incubated at 18±2°C. However, no appearance of disease symptoms was observed even on prolonged incubation.

From the results of experiments elaborated in the preceding pages, it can be clearly concluded that the explants obtained from the white rust induced hypertrophied inflorescence axis exhibit callus growth, which is much more rapid and profuse than that obtained from its healthy counterpart.

Microscopic examination of such a callus confirmed the presence of fungal structures of *A. candida* within it which was further examined by histopathological studies discussed later.
Table 9. Nature of response of different parts of *A. candida* induced hypertrophied inflorescence on the callusing medium after 5, 15 and 25 days.

<table>
<thead>
<tr>
<th>Explants</th>
<th>5 DAYS</th>
<th>15 DAYS</th>
<th>25 DAYS</th>
<th>Rating at 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Infected</td>
<td>Healthy</td>
<td>Infected</td>
</tr>
<tr>
<td>Flower bud</td>
<td>Slight thickening</td>
<td>Slight thickening</td>
<td>Poor callusing at cut ends</td>
<td>Poor callusing at cut ends</td>
</tr>
<tr>
<td>Sepals</td>
<td>Slight thickening</td>
<td>Slight thickening</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Petals</td>
<td>Slight thickening</td>
<td>Moderate thickening</td>
<td>Slight enlargement</td>
<td>Limited proliferation at cut ends</td>
</tr>
<tr>
<td>Anthers</td>
<td>No change</td>
<td>No change</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ovary</td>
<td>No change</td>
<td>Slight thickening</td>
<td>Thickening</td>
<td>Poor callusing at cut end</td>
</tr>
</tbody>
</table>
Table 9 contd.

<table>
<thead>
<tr>
<th>Explants</th>
<th>5 DAYS</th>
<th>15 DAYS</th>
<th>25 DAYS</th>
<th>Rating at 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickened apical leaf</td>
<td>Healthy</td>
<td>Infected</td>
<td>Healthy</td>
<td>Infected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickening at cut ends</td>
<td>Thickening</td>
<td>Proliferation</td>
<td>Rapid enlarge-</td>
<td>Callusing</td>
</tr>
<tr>
<td>Colour light green</td>
<td>and enlarge-</td>
<td>at cut ends</td>
<td>ment and</td>
<td>rooting and</td>
</tr>
<tr>
<td></td>
<td>ment. Colour</td>
<td></td>
<td>callusing.</td>
<td>shooting</td>
</tr>
<tr>
<td></td>
<td>light green.</td>
<td></td>
<td>Colour light</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>green.</td>
<td></td>
</tr>
<tr>
<td>Thickened No change apical</td>
<td>Pustule</td>
<td>No change</td>
<td>Yellowing of</td>
<td>No change</td>
</tr>
<tr>
<td>leaf with pustule</td>
<td>ruptures.</td>
<td></td>
<td>pustule region.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thickening</td>
<td></td>
<td>Enlargement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>enlargement.</td>
<td></td>
<td>continued.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colour light</td>
<td></td>
<td>Callusing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>green.</td>
<td></td>
<td>at cut ends.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Explants</th>
<th>5 DAYS</th>
<th>15 DAYS</th>
<th>25 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Infected</td>
<td>Healthy</td>
</tr>
<tr>
<td>Thickened Inflorescence</td>
<td>No change</td>
<td>Thickening prominent at cut ends. Pustule ruptured.</td>
<td>No change</td>
</tr>
</tbody>
</table>

Table 9 contd.

Plate 15. Growth response of infected and healthy inflorescence axis of cv. Pusa bold after 25 days.
Thus, infected callus obtained from hypertrophied inflorescence axis provided a perfect simplified system for the long term storage and maintainence of \textit{A. candida} under \textit{in vitro} conditions.

\textbf{Growth characteristics of \textit{A. candida} infected callus of cv. Pusa bold}

Growth response of healthy and \textit{A. candida} infected explant was compared on the callusing medium at 18±2°C. It was observed that proliferation of cut end cells of infected explants starts much earlier and their growth rate is much faster as compared to cell proliferation and growth rate exhibited by healthy explants.

Data on the comparative size and weight of both infected and healthy callus tissue after 15 days growth in callusing medium are presented in table 10 and Fig. 2. The entire body of the infected explant was converted into a large mass of callus tissue within 15 days of its transference into the callusing medium while the healthy explant only started callus initiation mainly from its cut end that was embedded into the medium during the same time interval (Ref. Plate 14).

However, the growth rate of the \textit{A. candida} infected callus started decreasing after this stage and on the 25\textsuperscript{th} day, the infected callus showed only a marginal increase in its size while the healthy explant maintained its growth and developed into a large callus mass during the period (Plate 15). With the passage of time, the callus gradually turned brown and finally dies.
Table 10. Average size and weight of healthy and *A. candida* infected callus tissue in callusing medium after 15 days at 18±2°C

<table>
<thead>
<tr>
<th>Nature of callus</th>
<th>INITIAL</th>
<th>AFTER 15 DAYS</th>
<th>PER CENT INCREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (mm)</td>
<td>Weight (g)</td>
<td>Size (mm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FW</td>
<td>DW</td>
</tr>
<tr>
<td>Healthy</td>
<td>7.5</td>
<td>0.028</td>
<td>0.002</td>
</tr>
<tr>
<td>Infected</td>
<td>26.5</td>
<td>0.066</td>
<td>0.006</td>
</tr>
</tbody>
</table>

FW - Fresh weight
DW - Dry weight
FIG. 2: PERCENT INCREASE IN SIZE (mm) AND WEIGHT (FRESH AND DRY) OF HEALTHY AND Albugo candido INFECTED CALLUS TISSUE AFTER 15 DAYS GROWTH AT 18±2°C
Maintenance of *A. candida* culture in its host callus under *in vitro* conditions

It can be seen from the above account that *A. candida* infected Brassica callus turns brown and eventually dies if it is maintained on the same medium for a prolonged period of about 25 days. Hence, the following two methods were tried for its maintainence.

i. Keeping an infected callus in juxtaposition with a healthy Brassica callus

Healthy, vigorously growing callus of cv. Pusa bold was raised in media tubes in the usual manner. When 15 days old, a piece of *A. candida* infected callus was aseptically introduced into each tube and placed in close contact with the healthy callus tissue. Such tubes were incubated at 18±2°C. At an interval of 7, 14 and 21 days, small pieces from the inoculated callus were removed and examined under the microscope for the presence of fungal structures but without any success.

The result shows that migration of the fungus *A. candida* does not take place from an infected callus to a healthy one when both are kept in close contact with each other.

ii. Subculturing of *A. candida* infected callus into a fresh medium

A 15 to 20 days old vigorously growing white callus infected with *A. candida* was chosen for the experiment. The infected callus was removed from the test tube into a sterile petridish and with the help of a scalpel cut into small pieces. These were then transferred
and placed partially embedded into fresh callusing medium in test tubes. The above procedure was carried out under sterile conditions in a laminar flow chamber. The inoculated test tubes were plugged and incubated at 18±2°C for the callus to grow.

Within 5 days, fresh white cells were seen developing from the subcultured callus body and soon rapid growth of the callus took place. These subcultured callus looked and behaved similarly to the original infected callus and when examined under the microscope, showed the presence of fungal mycelium within them. After their growth for 15-20 days on the medium, they were again transferred to a fresh medium for continued growth.

Thus, subculturing of *A. candida* infected callus proved to be a suitable method for the *in vitro* maintenance of dual culture of *A. candida* and its host.

**Effect of subculturing on the growth stage of**
*A. candida* **within its host callus**

The infected callus tissue was subcultured and maintained at 18±2°C as described before. At each subculture a portion of the callus which was being transferred was examined microscopically to confirm the presence of fungal hyphae within them.

After the callus had been maintained for about three to four months formation of the asexual stage was observed. Chains of sporangia were formed which were typically like those of *A. candida* found in nature. Details regarding the nature and position of the sporangia
are described under the histopathological studies of the infected tissue.

Effect of incubation temperature on the growth of *A. candida* within the host callus

To study the effect of temperature on the growth stage of *A. candida*, small pieces of vigorously growing infected callus were taken in media tubes and then incubated at 15, 20, 25 and 30°C for 20 days. After incubation, these were stained with cotton blue lactophenol and examined under the microscope.

Results showed that callus maintained at 15°C and 20°C contained only fungal mycelia and sporangia but those maintained at 25°C and 30°C showed the presence of both sporangia and oospores, the relative number of the latter being more in those callus that were maintained at 30°C. The experiment shows that *A. candida* can be induced to produce its asexual or sexual stage in its host callus tissue by varying the temperature of incubation.

Histopathological study of infected tissue

*A. candida* infected callus was embedded in wax, microtome sections were made and stained with cotton blue according to the method described earlier to examine the location and proliferation of the fungus within the callus tissue.

Examination of these microtome sections showed the presence of large thin-walled callus cells with only a few intercellular spaces between them. Abundant fungal mycelium were also observed which
Plate 16a,b Coenocytic intercellular mycelium of \textit{A. candida} in cv. Pusa bold callus.

Plate 17. Haustoria of \textit{A. candida} present within cv. Pusa bold callus cells.
were coenocytic and intercellular in nature (Plate 16). These hyphae
gave out small, more or less spherical haustoria which penetrated the
cell walls of the host tissue. Haustoria were connected to the mycelium
by a narrow stalk. Numerous haustoria were often observed to penetrate
a single cell of the callus tissue (Plate 17).

Asexual stage

When *A. candida* infected callus tissue was maintained
at 18±2°C for 3 to 4 months with subculturing interval of 20 days,
formation of sporangia was observed within the callus tissue. No
sporangia could be discerned on the surface of the callus mass but
examination of the sections of the tissue revealed the presence of several
sporangia within them. Sporangial chains frequently became dislocated
during section cutting but this was not unexpected because of the
unorganised nature of the soft callus. However, chains of sporangia could
be easily detected (Plate 18) which were similar to those found on the
host leaf (Plate 19).

The sporangial chains were borne by small mycelium which
were swollen at the ends (i.e. sporangiophores). The sporangia were
hyaline, globose or elliptical, thin walled and without any thickened bands.
These sporangial chains were formed away from the centre towards the
peripheral region but below the surface of the callus mass. The central
portion of the callus did not show the presence of any sporangia. The
sporangia were formed only in the vigorously growing whitish portions
of the callus and not in those parts which turned brown. The average
size of the sporangia varied between 20 to 16μ as compared to the
reported size of 12 x 23 x 12-20μ found in naturally infected host.
Plate 18. Sporangial chain of *A. candida* within the callus tissue of cv. Pusa bold.

Plate 20a. Oospore of *A. candida* within callus tissue of cv. Pusa bold.

Plate 20b. Oospore of *A. candida* found within callus tissue of cv. Pusa bold showing characteristic echinulations and thick wall layers.
Sexual stage

When the infected callus was incubated at temperatures between 24 to 30°C, numerous oospores embedded within the callus tissue including antheridia and oogonia were observed (Plate 19).

Oospore formed were typically like those of *A. candida*. They were globose, dark brown and highly differentiated having 5 layered cell wall. The epispore was thick having very prominent ridges (Plate 20). The oospore were 45 to 55 μ in size which is similar to the reported size of 40 to 60 μ, average 50 μ (Wagger, 1896). They were formed between the cells of the callus tissue, the cells being pushed backwards by the growing oogonium.

4.2.2 Dual culture of *P. parasitica* and its host

Downy mildew caused by *Peronospora parasitica* (Pers. ex. Fr.) Fr., is yet another important disease of rapeseed and mustard. It attacks all the aerial parts of the plant but dual infection of white rust and downy mildew, particularly in the hypertrophied tissue is of common occurrence.

Like *A. candida*, *P. parasitica* is also an obligate parasite which cannot be cultured and maintained on artificial media. Hence, attempts were made to establish *P. parasitica* on the callus tissue of *B. juncea* cv. Pusa bold.

Establishment of dual culture

To initiate dual culture, *P. parasitica* infected tissues of
of cv. Pusa bold and also of *P. parasitica* + *A. candida* infected tissue of the same were used as explants. They were surface sterilized and then aseptically transferred into the callusing medium tubes. Preparation of the medium and method of transfer were the same as described under materials and methods. The tubes were incubated at 18±2°C under low intensity white light.

Different types of explants were used for the initiation of dual culture of *P. parasitica* and its host and the results obtained from these experiments are as follows:

i. *P. parasitica* infected leaf pieces as explant

All the explants yielded various kinds of saprophytic fungi and bacteria leading to their death. Cent per cent contamination of explants may be due to their selection at an advanced stage of infection by the downy mildew fungus which favoured the invasion of other contaminants in their tissues.

ii. Leaf pieces from the margin of *P. parasitica* lesion as explant

Out of the explants introduced into the callusing medium, seventy five per cent showed only shrivelling after their transference into media tubes while the remaining ones yielded contaminant fungi and bacteria. These results indicate that Peronospora has a killing effect on the host tissues and thus prevents them from proliferating and growing into a callus.

From the above two experiments it can be concluded that Peronospora infected leaves are unsuitable for use as explant for the
establishment of dual culture.

iii. Leaf pieces with young unruptured white rust pustules

Such explants when placed on the callusing medium yielded the growth of *P. parasitica* from the area surrounding the white rust pustule in 16.66 per cent cases. Half of these (8.33%), however, showed the mixed growth of *P. parasitica* and a sterile fungus of unknown identity while the other half of the explants, yielded pure culture of the downy mildew fungus with abundant sporulation which ultimately covered their entire surface (Table 7).

It is evident from the above results that leaf tissues with unruptured pustule were invaded by *P. parasitica* at the early stage of white rust infection and the former showed sporulation after the explant was placed in the callusing medium. Callus formation started from the cut ends of these explants after about 4-5 days of their transfer into the medium but the growth was very restricted. However, the white rust pustule present on the explant surface showed no signs of further growth or maturity.

The explants which yielded mixed growth of *P. parasitica* and sterile fungal mycelium, ultimately died due to profuse growth of the latter.

Details regarding the growth of *P. parasitica* on the callus tissue have been provided while elaborating the characteristics of *in vitro* growth.
iv. \textit{Albugo candida} induced hypertrophied inflorescence axis pieces having the growth of on their surface

Culture of \textit{Peronospora} could also be obtained from \textit{A. candida} infected hypertrophied tissue. Success could not be achieved when the hypertrophied inflorescence axis used as explant was fully covered with the growth of downy mildew but those with only incipient growth of the pathogen, gave good results. Explants in the former case failed to exhibit any callus growth and yielded profuse growth of saprophytic fungi and bacteria. However, in the latter case, 14.28 per cent of the explants gave rise to \textit{P. parasitica} infected callus, 77.72% of them gave rise to only \textit{A. candida} infected callus devoid of any \textit{P. parasitica} while the remaining 8% died due to contamination.

These results show that explants from hypertrophied inflorescence axis with advanced stage of \textit{P. parasitica} infection are unsuitable for use as explants since they are highly prone to the invasion of saprophytic fungi and bacteria.

From the above two experiments it can be concluded that leaf explants with young unruptured white rust pustule as well as those obtained from white rust induced hypertrophied peduncle with incipient growth of \textit{P. parasitica}, exhibited enlargement and callus initiation besides supporting aerial growth of \textit{Peronospora} mycelium.

\textbf{Characteristics of \textit{P. parasitica} growth on \textit{A. candida} infected callus tissue}

Explants which yielded \textit{Peronospora}, showed aerial sporangiophores from their surface within 4-5 days of their introduction
Plate 21. Growth of *Peronospora parasitica* on *A. candida* infected callus of cv. Pusa bold after 10 days.

Plate 22. Growth of *Peronospora parasitica* on *A. candida* infected callus of cv. Pusa bold after 20 days.
Plate 23. Growth of *P. parasitica* on the callus tissue of cv. Pusa bold.

Plate 24. Sporangiophores and sporangia of *P. parasitica* on the callus tissue of cv. Pusa bold.
into the medium. The growth of the explant was slow but the fungus developed abundantly when incubated at 20±2°C in low intensity white light. After 15 days of incubation, the explant was converted into a callus mass, although callusing was not profuse. The growth of sporangiophores extended all over the callus (Plate 21).

However, such explants exhibited rapid senescence. First, the callus turned yellow from its original green colour but it remained turgid. Thereafter, it progressively turned brown and became flaccid till its death after about a month (Fig.3), but the fungus continued to sporulate until the eventual browning and death of the host callus (Plate 22). Microscopic examination confirmed typical colonies of *P. parasitica* (Plate 23) with sporangiophores and sporangia (Plate 24).

**Maintainence of *P. parasitica* culture**

Healthy leaf callus of cv. Pusa bold could be subcultured readily and these proliferated well on fresh medium. But due to rapid senescence of infected callus, the culture of *P. parasitica* could not be perpetuated by subculturing. The *in vitro* culture could be maintained, however, by keeping healthy or *A. candida* infected calluses in juxtaposition with a callus showing sporangial growth of *P. parasitica* on its surface and incubating them at 20±2°C with a drop of sterile distilled water.

Fungal hyphae from the infected callus invaded the new tissues after about 7-8 days and typical colony of *P. parasitica* with
FIG. 3: EFFECT OF INCUBATION PERIOD ON CALLUS FORMATION OF EXPLANT AND SPORULATION OF \textit{P. paragitica}
characteristic; sporangiophores and sporangia developed. The growth of the pathogen, however, always remained restricted to the callus tissue and the hyphae did not spread to the surrounding culture medium.

From the observation presented in the preceding pages it can be concluded that a dual culture of *P. parasitica* and its host cannot be established by using pre-infected tissue as explant but a culture of the pathogen *in vitro* could be obtained from *A. candida* infected host tissue showing the incipient growth of *P. parasitica*. Moreover, growth of *P. parasitica* on a callus lead to rapid death of the tissue while the presence of *A. candida* in the callus of cv. Pusa bold, enhanced its growth.

Subculturing of the *Peronospora* infected callus was not possible as the host callus aged rapidly. Perpetuation of the culture was possible only by keeping another callus in juxtaposition with an infected callus showing sporulation.

4.3 Biochemical analysis

4.3.1 Changes in biochemical constituents and mineral contents in inflorescence of cv. Pusa bold with the progress of white rust disease

Results presented herein pertain to investigations carried out to determine the changes that occur in the concentration of reducing and non-reducing sugars, total free amino acids, total phenols, crude protein, and per cent moisture and mineral content of white rust infected inflorescence of cv. Pusa bold with the progress of the disease.

Floral malformation was induced by inoculating 60 days old
Plate 25. Three stages in the development of *A. candida* induced hypertrophy.

a. Initial stage
b. Middle stage
c. Final stage
cv. Pusa bold plants with a sporangial suspension of *A. candida* and three stages in the process of malformation of the inflorescence (Plates 25a, b and c) viz., (a) Initial stage - tissue swelling incipient, (b) Middle stage - tissue swelling pronounced and (c) Final stage - distortion and malformation complete, were chosen for analysis.

The experiment was carried out in triplicate and only their average value was considered.

**Sugars**

White rust infection profoundly affects the normal physiological activities of the plant leading to abnormal swelling and distortion of its inflorescence axis and other floral organs. Hence, the possibility of a gradual change in the concentration of reducing and non-reducing sugars in the infected inflorescence with the progress of the white rust disease was investigated.

**Reducing sugars**

Significant changes in the concentration of reducing sugars was observed in the inflorescence with the progress of the white rust disease (Table 11 and Fig. 4).

The inflorescence with incipient swelling showed 8.36 mg/g of reducing sugars. The concentration of reducing sugars increased rapidly with the progress of infection and at the middle stage of malformation, when swelling and distortion became prominent, the sugar content in the infected host tissue increased to 12.76 mg/g, signifying
Table 11. Biochemical constituents of white rust infected inflorescence of cv. Pusa bold at three stages of its malformation (Average values in mg/gm fresh weight)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>INITIAL STAGE</th>
<th>MIDDLE STAGE</th>
<th>FINAL STAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average value</td>
<td>Average value</td>
<td>Average value</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>8.36</td>
<td>12.76</td>
<td>+52.63</td>
</tr>
<tr>
<td>Non Reducing sugars</td>
<td>15.52</td>
<td>20.363</td>
<td>+31.20</td>
</tr>
<tr>
<td>Phenols</td>
<td>0.365</td>
<td>0.180</td>
<td>-50.68</td>
</tr>
<tr>
<td>Total free Aminoacids</td>
<td>12.00</td>
<td>12.45</td>
<td>+3.75</td>
</tr>
<tr>
<td>% Moisture content</td>
<td>79.12</td>
<td>81.96</td>
<td>+3.59</td>
</tr>
</tbody>
</table>
an increase of 52.63 per cent over that of the initial stage. Thereafter, there was practically no further increase in the concentration of reducing sugars since the fully malformed inflorescence showed a concentration of 12.82 mg/g, an increase of only 0.0047 per cent over that of the middle stage.

These results show that systemic infection of inflorescence by *A. candida* causes rapid increase in its reducing sugar content and the maximum increase takes place between the initial and middle stage of hypertrophy.

**Non-reducing sugars**

The results obtained show that the concentration of non-reducing sugars in the infected inflorescence also increased with the progress of the white rust disease.

During the initial stage, the inflorescence with incipient swelling showed a concentration of 15.52 mg/g. The highest increase in non-reducing sugar content was detected in inflorescence at the middle stage of malformation process with prominent swelling. It recorded a concentration of 20.36 mg/g, which is 31.20 per cent more than that in the initial stage. Accumulation of sugars in the host tissue continue to increase with age, the extent of increase being slightly lower in the final stage of malformation, when the infected tissue showed a concentration of 23.91 mg/g, an increase of 17.43 per cent over the previous stage.

Thus, a gradual increase in non-reducing sugar content was
observed as the inflorescence became progressively malformed and the changes in concentration became conspicuous between the initial and middle stages of hypertrophy.

**Phenols**

The data presented in Table 11 and Fig. 4 show a decrease in phenol content in the infected inflorescence with the progress of white rust infection.

The concentration of phenols at the initial stage of infection was 0.365 mg/g fresh weight of infected tissue. As the infection progressed and the distortion became prominent at the mid stage, the phenol content of the infected tissue stood at 0.180 mg/g, recording a sharp decline of 50.68 per cent while at the final stage of malformation, the host tissue indicated a further decline of 39.44 per cent in its phenol content.

**Total free amino acids**

Changes in the concentration of total free amino acids in different stages of floral hypertrophy was estimated according to the method stated earlier.

An appraisal of the data presented in table 11 and Fig. 4 shows that the concentration of total free amino acid increased gradually from the initial to the final stage of malformation process.

At the initial stage, the concentration of total free amino acids was 12.00 mg/g of malformed tissue. During the middle and final
FIG. 4: SUGARS, TOTAL FREE AMINO ACIDS, CRUDE PROTEIN, PHENOLS AND MOISTURE CONTENT OF WHITE RUST INFECTED INFLORESCENCE OF cv. PUSA BOLD AT THREE STAGES IN THE DEVELOPMENT OF HYPERTROPHY
stage of malformation, the concentration of amino acids was respectively recorded as 12.45 mg/g and 12.72 mg/g indicating an increase of 3.75 per cent and 2.17 per cent respectively over their respective previous stage.

Crude protein

Studies on the crude protein content of infected tissue also showed a significant decrease in its concentration with the progress of white rust infection (Table 11 and Fig. 4).

At the initial stage of hypertrophy, the crude protein content of inflorescence was estimated at 9.06 mg/g of host tissue. Its concentration stood at 7.13 mg/g at the mid stage of malformation registering a decline of 21.30 per cent while at the final stage, the concentration declined by another 9.39 per cent over the middle stage.

From the data presented above, it is evident that white rust infection causes a decrease in crude protein content of inflorescence tissue and the decrease was observed to be maximum between the initial and middle stage of malformation process.

Moisture content

The data presented in table 11 and Fig. 4 show that at the onset of the thickening process, the moisture content of inflorescence showing incipient swelling was 79.12 per cent.

An increase in moisture content by 3.59 per cent over the
initial stage took place as the thickening became prominent in the middle stage. Thereafter, its concentration in infected tissue decreased as the hypertrophy matured and the moisture content of hypertrophied inflorescence at the final stage showed a decrease of 5.38 per cent over that of the middle stage.

Nitrogen

The nitrogen content of inflorescence declined as a result of infection with white rust (Table 12 and Fig. 5). At the initial stage of malformation, the concentration of nitrogen was 14.52 mg/g but at the middle and final stage, its concentration in infected inflorescence declined respectively, by 21.42 per cent and 9.37 per cent indicating highest decline between the initial and middle stage of the malformation process.

Phosphorus

The data presented in table 12 and Fig. 5 show that the phosphorus content of inflorescence with incipient malformation was 2.60 µg/g of fresh tissue weight. At the middle stage of malformation, the concentration of phosphorus declined by 16.92 per cent to 2.16 µg/g but thereafter, its concentration increased by 17.59 per cent to 2.54 µg/g at the completely mature staghead stage.

Potassium

Infection with the fungus lead to a net increase in potassium content of the inflorescence as is evident from the data furnished in table 12 and Fig. 5.
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Initial Stage</th>
<th>Middle Stage</th>
<th>Final Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>14.52</td>
<td>11.41</td>
<td>-9.37</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.60</td>
<td>2.16</td>
<td>+17.59</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.53</td>
<td>3.85</td>
<td>+36.36</td>
</tr>
</tbody>
</table>
FIG. 5: NITROGEN PHOSPHORUS AND POTASSIUM CONTENT OF WHITE RUST INFECTED INFLORESCENCE OF cv. PUSA BOLD AT THREE STAGES IN THE DEVELOPMENT OF HYPERTROPHY.
A potassium content of 4.53 μg/g dry weight host tissue was observed at the onset of the thickening process in the inflorescence. A decline in its concentration (3.85 μg/g) occurred during the middle stage but its concentration again showed an increase by 36.36 per cent to 5.25 μg/g at the final stage of staghead formation.

Cu, Fe, Mn, Zn, Pb, Cd and Co

The data presented in table 13 and Fig. 6 show that the concentration of Cu, Fe, Mn and Zn declined in the inflorescence of cv. Pusa bold as a result of hypertrophy formation due to white rust infection. The change in the concentration of Pb, Cd and Co was not determined as they were present in traces only.

The Cu content was 56.56 μg/g dry weight in inflorescence at the incipient malformation stage. Its concentration decreased by 42.86 per cent to 32.32 μg/g dry weight as the hypertrophy become prominent in the middle stage. As the malformation process progressed, the Cu content again increased by 0.5 per cent over the middle stage to a concentration of 48.48 μg/g at the fully mature staghead stage.

The Fe content of white rust infected inflorescence decreased by 8.20 per cent from 685.39 μg/g to 629.21 μg/g dry weight host tissue as the disease progressed from the initial to the middle stage of malformation. The Fe content decreased further with the progress of the disease to a concentration of 460.67 μg/g in the fully mature staghead stage indicating a decline of 26.78 per cent over the middle stage.

Similar trend of decline was also noted in respect to the concentration of both Mn and Zn in inflorescence following infection with
Table 13. Copper, Iron, Manganese and Zinc content of white rust infected inflorescence of cv. Pusa bold at three stages of its malformation (Average values in ug/g dry weight)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>INITIAL STAGE</th>
<th>MIDDLE STAGE</th>
<th>FINAL STAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average value</td>
<td>Average value</td>
<td>% change over initial stage</td>
</tr>
<tr>
<td>Cu</td>
<td>56.56</td>
<td>32.32</td>
<td>-42.86</td>
</tr>
<tr>
<td>Mn</td>
<td>72.26</td>
<td>61.94</td>
<td>-14.28</td>
</tr>
<tr>
<td>Fe</td>
<td>685.39</td>
<td>629.21</td>
<td>-8.20</td>
</tr>
<tr>
<td>Zn</td>
<td>10.96</td>
<td>6.81</td>
<td>-37.86</td>
</tr>
</tbody>
</table>

Pb, Cd and Co were present in trace.
FIG. 6: COPPER, MANGANESE, IRON AND ZINC CONTENT IN WHITE RUST INFECTED INFLORESCENCE OF cv. PUSA BOLD AT THREE STAGES IN THE DEVELOPMENT OF HYPERTROPHY
white rust.

The Mn content was 72.26 µg/g in inflorescence at its incipient swelling stage. Thereafter, its concentration stood at 61.94 µg/g during the middle stage and 46.45 µg/g during the fully malformed stage showing respectively, 14.28 per cent and 25.00 per cent decline over their respective preceding stage.

The concentration of Zn was 10.96 µg/g dry weight during the initial stages of inflorescence thickening and then decreased by 37.86 per cent as the thickening became prominent in the middle stage. After this as the staghead became mature, the Zn content of the infected tissue was found to be 5.89 µg/g dry weight, indicating a further decline of 13.51 per cent over that of the middle stage.

Thus, from the above results it can be concluded that the concentration of Cu, Fe, Mn and Zn decreased with the progress of white rust disease in the inflorescence of cv. Pusa bold. In case of Cu and Zn, the maximum decrease in concentration was observed between the initial and middle stages of hypertrophy formation while in case of Fe and Mn, conspicuous decrease was evident between the middle and final stages.

4.3.2 Physiology of disease resistance

Existence of possible relationship between reducing and non-reducing sugars, total free amino acids, phenols and per cent moisture and minerals content of the two *B. juncea* cvs. Pusa bold (susceptible) and Bio YSR (resistant) to their reaction to white rust infection was examined in the present study.
Leaves and young flowering twigs of 60 days old plants of cv. Pusa bold were uniformly inoculated with the sporangial suspension of *A. candida*. Uninoculated healthy plants of cv. Pusa bold as well as those of cv. Bio YSR, which do not take up infection, were used as control for comparison.

After 20 days following inoculation, the 5th bottom leaf from both diseased and healthy plants of the above cultivars were separately collected for analysis. Similarly, hypertrophied inflorescence from the inoculated cv. Pusa bold plants as well as healthy inflorescence of equivalent age from both cvs. Pusa bold and Bio YSR were harvested for the above study. All experiments were carried out in triplicate and their average value was considered.

### Sugars

It is a well established fact that changes in nitrogen metabolism, enzyme activities and other biochemical processes arising from infection by a pathogen results in changes in the respiratory as well as photosynthetic rate of the infected plant. This in turn disturbs the concentration of sugars and other carbohydrates in such plants. Therefore, the concentration of reducing and non-reducing sugars in the leaves of white rust susceptible and resistant cultivars were determined.

#### Reducing sugars

Data presented in table 14 and Fig. 7 show that inflorescence contain more reducing sugars than the leaves. Further, the reducing sugar content of healthy leaves and inflorescence of the
Table 14. Biochemical constituents of two B. juncea cvs. Pusa bold (susceptible) and Bio YSR (resistant) before and after infection with A. candida (Average values in mg/g fresh weight)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>PUSA BOLD</th>
<th>Bio YSR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Inflorescence</td>
</tr>
<tr>
<td></td>
<td>Healthy Infected</td>
<td>% increase (+) decrease (-) over healthy</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>3.30</td>
<td>2.70</td>
</tr>
<tr>
<td>Non Reducing sugars</td>
<td>10.25</td>
<td>10.00</td>
</tr>
<tr>
<td>Phenols</td>
<td>0.28</td>
<td>0.134</td>
</tr>
<tr>
<td>Total free Aminoacids</td>
<td>7.97</td>
<td>10.46</td>
</tr>
<tr>
<td>Crude protein</td>
<td>13.76</td>
<td>12.95</td>
</tr>
<tr>
<td>% Moisture content</td>
<td>79.02</td>
<td>77.82</td>
</tr>
</tbody>
</table>
FIG. 7: REDUCING SUGAR, NONREDUCING SUGAR AND % MOISTURE CONTENT OF TWO B. JUNCEA cvs PUSA BOLD (SUSCEPTIBLE) AND BIO YSR (RESISTANT) BEFORE AND AFTER INFECTION WITH A. candida
resistant cv. Bio YSR was higher as compared to its concentration in their healthy counterparts of the susceptible cv. Pusa bold.

In case of the susceptible cultivar, the reducing sugar content of leaves decreased by 18.18 per cent while that of inflorescence increased by as much as 132.58 per cent due to infection with white rust as compared to their healthy counterparts.

Non-reducing sugar

The two cultivars also differed in the concentration of non-reducing sugars. Its concentration in leaves and inflorescence of the immune cultivar was higher than that of the susceptible cultivar.

Due to infection with *A. candida*, a decrease in non-reducing sugar content by 2.44 per cent was noted in the infected leaves of the susceptible cultivar. On the other hand, the concentration of non-reducing sugars increased in the infected inflorescence by 82.80 per cent over its healthy tissue.

Above results show that the concentration of sugars in the resistant cultivar is more than the susceptible cultivar both in leaves and inflorescence. Concentration of sugars decreases in leaves but increases in inflorescence after infection with the white rust disease.

Phenols

The concentration of total phenols in the resistant and the susceptible cultivar was estimated and also the change in its total content after infection with *A. candida* in the susceptible cultivar was
determined (Table 14 and Fig. 8). The leaves and inflorescence of the resistant cultivar contained more concentration of total phenols as compared to their healthy counterparts of the susceptible plant.

Following infection with *A. candida*, the total phenol content decreased by 52.14 per cent and 64.15 per cent respectively, in leaves and inflorescence of the susceptible cultivar.

**Total free amino acids**

An appraisal of data presented in table 14 Fig. 8 shows a difference in the concentration of total free amino acids between the resistant and susceptible cultivars.

Higher concentration of total free amino acids was observed in the healthy leaves and inflorescence of the susceptible cultivar as compared to those of the resistant cultivar. Also as a result of infection by *A. candida* the susceptible cultivar showed an increase in the concentration of free amino acids, the extent of increase being 31.24 per cent and 4.18 per cent respectively, in case of leaves and inflorescence.

**Crude protein**

Data presented in table 14 and Fig. 8 show that unlike total phenols, the crude protein content of both leaves and inflorescence of the resistant cv. Bio YSR was lower as compared to its concentration in their healthy counterparts of the susceptible cv. Pusa bold.

In the susceptible cultivar, due to infection by *A. candida*
FIG. 8: PHENOLS, AMINO ACIDS AND CRUDE PROTEIN CONTENT OF TWO \textit{B. JUNCEA} cvs PUSA BOLD (SUSCEPTIBLE) AND BIO YSR (RESISTANT) BEFORE AND AFTER INFECTION WITH \textit{A. candida}
a decrease in crude protein content was observed. The concentration decreased by 5.89 per cent in leaves and by 14.08 per cent in inflorescence.

Moisture content

A study of data presented in table 14 and Fig. 7 show that the moisture content of the healthy leaves and inflorescence in both the cultivars was almost similar.

However, due to infection of the susceptible cultivar by *A. candida* its moisture content decreased by 1.52 per cent in leaves but increased by 5.73 per cent in inflorescence.

Nitrogen

The susceptible cultivar showed a higher concentration of nitrogen in its leaves and inflorescence as compared to its resistant counterpart (Table 15 and Fig. 9). However, the nitrogen content of the susceptible cv. Pusa bold after infection with *A. candida* decreased by 6.28 per cent in leaves but increased by 29.87 per cent in inflorescence.

Phosphorus

An appraisal of the data presented in the table 15 and Fig. 9 show that the phosphorus content of healthy leaves and inflorescence of the two cultivars were almost similar.

In case of the susceptible cultivar, a net decrease in phosphorus content was observed after infection with *A. candida*, the decrease being
Table 15.
Concentration of Nitrogen, Phosphorus, and Potassium in two B. juncea cvs. Pusa bold (susceptible) and Bio YSR (resistant) before and after infection with A. candida (average values in µg/g dry weight).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Healthy</th>
<th>Infected</th>
<th>% Increase (+)/Decrease (-) Over Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>2.07</td>
<td>2.31</td>
<td>-6.28</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.21</td>
<td>0.17</td>
<td>-18.04</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.39</td>
<td>0.43</td>
<td>+10.26</td>
</tr>
</tbody>
</table>

Bioc, YSR
Leaf Inflorescence
Pusa bold
FIG. 9: NITROGEN, PHOSPHORUS AND POTASSIUM CONTENT OF TWO B. JUNCEA cvs PUSA BOLD (SUSCEPTIBLE) AND BIO YSR (RESISTANT) BEFORE AND AFTER INFECTION WITH A. candida
Fig. 10: Copper, manganese, zinc and iron content in two B. juncea cvs PUSA Bold (susceptible) and Bio YSR (resistant) before and after infection with A. candida.
19.04 per cent in case of leaves and 14.70 per cent in case of inflorescence.

Potassium

Data presented in table 15 and Fig. 9 show a slightly higher content of potassium in both leaves and inflorescence of the susceptible cv. Pusa bold as compared to its concentration in the resistant cv. Bio YSR. The potassium content of the susceptible cultivar also increased significantly after infection with white rust, the extent of increase being 10.26 per cent in leaves and 62.5 per cent in inflorescence.

Cu, Fe, Mn and Zn

Concentration of these minerals was estimated according to the method stated earlier and the results are presented in table 16 and Fig. 10.

Concentration of copper in both leaves and inflorescence of the resistant cultivar was higher than its concentration in both healthy leaves and inflorescence of the susceptible cultivar. Further, the susceptible cultivar showed a decrease in its copper content after infection with white rust, the decrease in concentration being 2.14 per cent in leaves and as high as 32.75 per cent in case of inflorescence.

Like copper, iron content was also higher in the resistant cultivar as compared to its concentration in the susceptible cultivars, its concentration being more in leaves than in inflorescence in both the cultivars. However, iron content decreased by 6.69 per cent in
Table 16. Concentration of Copper, Iron, Manganese and Zinc content in two *B. juncea* cvs. Pusa bold (Susceptible) and Bio YSR (resistant) before and after infection with (Average values in μg/g dry weight)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Pusa bold</th>
<th>Bio YSR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Inflorescence</td>
</tr>
<tr>
<td></td>
<td>Healthy % decrease over healthy</td>
<td>Infected</td>
</tr>
<tr>
<td>Copper</td>
<td>45.78 44.8 -2.14</td>
<td>62.6 42.1 -32.75</td>
</tr>
<tr>
<td>Iron</td>
<td>1063.67 992.5 -6.69</td>
<td>929.21 561.8 -39.54</td>
</tr>
<tr>
<td>Manganese</td>
<td>95.48 90.32 -5.40</td>
<td>90.07 49.5 -45.04</td>
</tr>
</tbody>
</table>

Ph, Cd and Co were present in traces only.
leaves and by 39.54 per cent in inflorescence of the susceptible cv. Pusa bold after infection with *A. candida*.

Manganese concentration in the resistant cv. Bio YSR was against as compared to its concentration in the susceptible cv. Pusa bold. However, infection with *A. candida* caused a decrease in manganese content of the susceptible cultivar the decrease being 5.40 per cent in leaves and 45.04 per cent in inflorescence.

In contrast to Cu, Fe and Mn, the concentration of Zn in the resistant cultivar was found to be lower as compared to its concentration in the susceptible cultivar. Further, the concentration of zinc in inflorescence was found to be higher than its concentration in leaves of both the cultivars.

Data presented in table 16 and Fig. 10 also show, that the susceptible cultivar suffered a significant decrease in its zinc content after infection with white rust, the decrease being 38.65 per cent in case of leaves and 66.62 per cent in case of inflorescence.

From the above results, it can be concluded that the concentration of copper, iron and manganese was more in the resistant cultivar as compared to its concentration in the susceptible cultivar while the opposite was true in case of zinc. Concentration of these elements decreases in plants due to infection with *A. candida*. 
4.4 Chemical control

The present field experiment was conducted during the years 1988-89 and 1989-90 in an attempt to develop an economic spray schedule for the control of white rust and Alternaria blight diseases. A total of six fungicides, comprising three systemic and three non-systemic, were sprayed at varying time intervals in the manner stated earlier. The three systemic fungicides used were Oxadixyl, Ridomil and Topsin while the nonsystemic were Dithane M-45, Rovral and Chlorothalonil.

Data presented in Tables 17 and 18 and Figs. 11 and 12 indicate that the incidence of both the disease, as such was considerably more during the year 1988-89 than 1989-90. In general, application of either a systemic or non-systemic fungicide considerably reduced the intensity of white rust as well as that of Alternaria blight. However, among the various systemic fungicides Ridomil was the most effective followed by Oxadixyl and Topsin. Increasing number of sprays of these fungicides progressively and significantly reduced the incidence of both the diseases. In case of Ridomil results were significant even with two sprays but in the case of others significant decrease in disease intensity could be achieved with at least three sprays. In all cases, it was, however, found that there was no significant difference in effects between three and four sprays and therefore, only three sprays were found to be sufficient for an effective control of the two diseases. Irrespective of the number of sprays applied, the effectiveness of Ridomil proved to be highest during both the years in decreasing the disease intensity. This was found to be true for both the diseases.
Table 17. Comparative effect of systemic and nonsystemic fungicides on the incidence of Alternaria blight of rapeseed and mustard.

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Disease</td>
<td>% decrease</td>
<td>% Disease</td>
<td>% decrease</td>
</tr>
<tr>
<td></td>
<td>intensity</td>
<td>over control</td>
<td>intensity</td>
<td>over control</td>
</tr>
<tr>
<td>Oxadixyl (one spray)</td>
<td>30.36</td>
<td>-10.71</td>
<td>6.7</td>
<td>-22.09</td>
</tr>
<tr>
<td></td>
<td>33.43</td>
<td></td>
<td>14.83</td>
<td></td>
</tr>
<tr>
<td>Oxadixyl (two sprays)</td>
<td>25.3</td>
<td>-25.59</td>
<td>6.53</td>
<td>-24.06</td>
</tr>
<tr>
<td></td>
<td>30.11</td>
<td></td>
<td>14.88</td>
<td></td>
</tr>
<tr>
<td>Oxadixyl (three sprays)</td>
<td>19.06</td>
<td>-43.94</td>
<td>5.16</td>
<td>-40.00</td>
</tr>
<tr>
<td></td>
<td>25.89</td>
<td></td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Oxadixyl (four sprays)</td>
<td>17.7</td>
<td>-47.94</td>
<td>4.2</td>
<td>-51.16</td>
</tr>
<tr>
<td></td>
<td>24.87</td>
<td></td>
<td>11.87</td>
<td></td>
</tr>
<tr>
<td>Ridomil (one spray)</td>
<td>28.13</td>
<td>-17.26</td>
<td>6.53</td>
<td>-24.06</td>
</tr>
<tr>
<td></td>
<td>32.01</td>
<td></td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>Ridomil (two sprays)</td>
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<td>-49.41</td>
<td>4.93</td>
<td>-57.32</td>
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<tr>
<td></td>
<td>25.18</td>
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<td>12.79</td>
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</tr>
<tr>
<td>Ridomil (three sprays)</td>
<td>9.31</td>
<td>-72.61</td>
<td>2.93</td>
<td>-65.93</td>
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<tr>
<td></td>
<td>17.59</td>
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<td>9.85</td>
<td></td>
</tr>
<tr>
<td>Ridomil (four sprays)</td>
<td>7.5</td>
<td>-77.94</td>
<td>3.00</td>
<td>65.12</td>
</tr>
<tr>
<td></td>
<td>15.82</td>
<td></td>
<td>9.98</td>
<td></td>
</tr>
<tr>
<td>Topsin (one spray)</td>
<td>29.83</td>
<td>-12.26</td>
<td>8.06</td>
<td>-6.23</td>
</tr>
<tr>
<td></td>
<td>33.07</td>
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<td>16.4</td>
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<tr>
<td>Topsin (two sprays)</td>
<td>27.36</td>
<td>-19.53</td>
<td>6.7</td>
<td>-22.09</td>
</tr>
<tr>
<td></td>
<td>31.52</td>
<td></td>
<td>14.97</td>
<td></td>
</tr>
<tr>
<td>Topsin (three sprays)</td>
<td>21.7</td>
<td>-36.17</td>
<td>6.36</td>
<td>-26.04</td>
</tr>
<tr>
<td></td>
<td>27.71</td>
<td></td>
<td>14.59</td>
<td></td>
</tr>
<tr>
<td>Topsin (four sprays)</td>
<td>17.96</td>
<td>-47.17</td>
<td>5.16</td>
<td>-40.00</td>
</tr>
<tr>
<td></td>
<td>25.01</td>
<td></td>
<td>13.09</td>
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</tr>
<tr>
<td>Chlorothalonil</td>
<td>23.23</td>
<td>-31.67</td>
<td>5.96</td>
<td>-30.69</td>
</tr>
<tr>
<td></td>
<td>28.83</td>
<td></td>
<td>14.06</td>
<td></td>
</tr>
<tr>
<td>Rovral (two sprays)</td>
<td>14.66</td>
<td>-56.88</td>
<td>5.46</td>
<td>-36.5</td>
</tr>
<tr>
<td></td>
<td>22.34</td>
<td></td>
<td>13.51</td>
<td></td>
</tr>
<tr>
<td>Dithane M45</td>
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<td>3.8</td>
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</tr>
<tr>
<td></td>
<td>18.74</td>
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<td>11.21</td>
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<tr>
<td>Control</td>
<td>34.0</td>
<td>0</td>
<td>8.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>35.58</td>
<td></td>
<td>16.94</td>
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</table>

C.D. at 5% 4.98 2.30

Figures in parenthesis denote angular transformed values.
Table 18. Comparative effect of systemic and nonsystemic fungicides on the incidence of white rust of rapeseed and mustard.

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</thead>
<tbody>
<tr>
<td></td>
<td>% Disease intensity</td>
<td>% decrease over control</td>
<td>% Disease intensity</td>
<td>% decrease over control</td>
</tr>
<tr>
<td>Oxadixyl</td>
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<td>-21.15</td>
<td>8.33</td>
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<tr>
<td>(one spray)</td>
<td>(34.93)</td>
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<td>(16.76)</td>
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<td>30.3</td>
<td>-27.16</td>
<td>8.2</td>
<td>-28.88</td>
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<td>(two sprays)</td>
<td>(33.39)</td>
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<td>(16.66)</td>
<td></td>
</tr>
<tr>
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<td>-37.98</td>
<td>4.3</td>
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<tr>
<td>(three sprays)</td>
<td>(30.51)</td>
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<td>(11.96)</td>
<td></td>
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<tr>
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<td>-63.22</td>
<td>4.23</td>
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<tr>
<td>(four sprays)</td>
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<td>(11.77)</td>
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<tr>
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<tr>
<td>(one spray)</td>
<td>(33.1)</td>
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<tr>
<td>(two sprays)</td>
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<td>(three sprays)</td>
<td>(10.88)</td>
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<td>(10.68)</td>
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<td>Ridomil</td>
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<td>3.96</td>
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<tr>
<td>(four sprays)</td>
<td>(9.35)</td>
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<td>(11.46)</td>
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<tr>
<td>Topsin</td>
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<td>-20.12</td>
<td>8.9</td>
<td>-22.81</td>
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<tr>
<td>(one spray)</td>
<td>(35.19)</td>
<td></td>
<td>(17.31)</td>
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<tr>
<td>Topsin</td>
<td>30.46</td>
<td>-26.77</td>
<td>7.96</td>
<td>-30.96</td>
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<tr>
<td>(two sprays)</td>
<td>(33.5)</td>
<td></td>
<td>(16.35)</td>
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</tr>
<tr>
<td>Topsin</td>
<td>22.37</td>
<td>-46.22</td>
<td>6.9</td>
<td>-40.15</td>
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<td>(three sprays)</td>
<td>(28.19)</td>
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<td>(15.20)</td>
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<tr>
<td>Topsin</td>
<td>20.13</td>
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<td>6.43</td>
<td>-44.23</td>
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<td>(four sprays)</td>
<td>(26.6)</td>
<td></td>
<td>(14.64)</td>
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<tr>
<td>Chlorothalonil</td>
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<td>5.5</td>
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<td>(28.96)</td>
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<td></td>
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<td>Rovral</td>
<td>27.33</td>
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<td>6.06</td>
<td>-47.44</td>
</tr>
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<td>(31.41)</td>
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<td>(14.24)</td>
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<tr>
<td>Dithane M45</td>
<td>14.4</td>
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<td>4.00</td>
<td>65.31</td>
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<tr>
<td>(22.26)</td>
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<td></td>
<td>(11.54)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>0</td>
<td>11.53</td>
<td>0</td>
</tr>
<tr>
<td>(40.16)</td>
<td></td>
<td></td>
<td>(19.76)</td>
<td></td>
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C.D. at 5% 6.22 2.34

Figures in parenthesis denote angular transformed values.
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<thead>
<tr>
<th>Fungicides</th>
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<tr>
<td><strong>Non Systemic</strong></td>
<td></td>
<td>Four sprays each.</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td>Chlorothalonil</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td></td>
<td>Rovral</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td></td>
<td>Dithane M-45</td>
</tr>
</tbody>
</table>

| **Systemic**               |   |               |
| **O₁**                     |   | Oxadixyl 1 spray |
| **O₂**                     |   | Oxadixyl 2 sprays |
| **O₃**                     |   | Oxadixyl 3 sprays |
| **O₄**                     |   | Oxadixyl 4 sprays |
| **RD₁**                    |   | Ridomil 1 spray |
| **RD₂**                    |   | Ridomil 2 sprays |
| **RD₃**                    |   | Ridomil 3 sprays |
| **RD₄**                    |   | Ridomil 4 sprays |
| **T₁**                     |   | Topsin 1 spray |
| **T₂**                     |   | Topsin 2 sprays |
| **T₃**                     |   | Topsin 3 sprays |
| **T₄**                     |   | Topsin 4 sprays |
FIG. 11: COMPARATIVE EFFECT OF SYSTEMIC AND NONSYSTEMIC FUNGICIDES ON THE INCIDENCE OF ALTERNARIA BLIGHT OF RAPESEED AND MUSTARD
FIG. 12: COMPARATIVE EFFECT OF SYSTEMIC AND NONSYSTEMIC FUNGICIDES ON THE INCIDENCE OF WHITE RUST OF RAPESEED AND MUSTARD
Among the three non systemic fungicides, Dithane M-45 decreased the disease intensity most as compared to control for both the diseases during 1988-89 as well as 1989-90. However, the results were significant for all the three fungicides under this group.

For the control of Alternaria blight, Chlorothalonil was least effective and the effects of Dithane M 45 and Rovral were significantly higher over this fungicide. But no significant difference was observed between Rovral and Chlorothalonil for the control of white rust wherein Dithane M-45 was found to be significantly better than both of these fungicides.

Between the systemic and non-systemic fungicides, the effectiveness of the former was consistently better in decreasing the intensity of both the diseases studied here.

Data on seed yield (Table 19 and Fig. 13) revealed an improvement with the application of systemic as well as non-systemic fungicides but was markedly more under the former as compared with the latter. Although, increasing number of sprays progressively increased the seed yield, the differences were significant with four sprays of Oxadiyl as against only three sprays of Ridomil during the year 1988-89. The effect of Topsin was not significant on yield under any one of the spray schedule. However, during the year 1989-90 only two sprays of Ridomil or three sprays of Oxadiyl increased the yield significantly over control. Again the effect of Topsin was found to be non significant.
Table 19. Comparative effect of systemic and nonsystemic fungicides on the seed yield of rapeseed and mustard.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Yield in Kg/ha</td>
<td>% increase over control</td>
</tr>
<tr>
<td>Oxadixyl (one spray)</td>
<td>591.66</td>
<td>5.18</td>
</tr>
<tr>
<td>Oxadixyl (two sprays)</td>
<td>666.6</td>
<td>18.50</td>
</tr>
<tr>
<td>Oxadixyl (three sprays)</td>
<td>687.5</td>
<td>22.22</td>
</tr>
<tr>
<td>Oxadixyl (four sprays)</td>
<td>712.5</td>
<td>26.66</td>
</tr>
<tr>
<td>Ridomil (one spray)</td>
<td>595.83</td>
<td>6.92</td>
</tr>
<tr>
<td>Ridomil (two sprays)</td>
<td>670.83</td>
<td>19.25</td>
</tr>
<tr>
<td>Ridomil (three sprays)</td>
<td>829.16</td>
<td>47.40</td>
</tr>
<tr>
<td>Ridomil (four sprays)</td>
<td>883.33</td>
<td>57.03</td>
</tr>
<tr>
<td>Topsin (one spray)</td>
<td>587.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Topsin (two sprays)</td>
<td>658.33</td>
<td>17.03</td>
</tr>
<tr>
<td>Topsin (three sprays)</td>
<td>683.33</td>
<td>21.48</td>
</tr>
<tr>
<td>Topsin (four sprays)</td>
<td>687.5</td>
<td>22.22</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>675.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Rovral</td>
<td>633.33</td>
<td>12.58</td>
</tr>
<tr>
<td>Dithane M45</td>
<td>714.58</td>
<td>27.03</td>
</tr>
<tr>
<td>Control</td>
<td>562.5</td>
<td>0</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>139.22</td>
<td>109.07</td>
</tr>
</tbody>
</table>
### Fungicides

<table>
<thead>
<tr>
<th>Non Systemic</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Four sprays each.</td>
<td></td>
</tr>
<tr>
<td>Chlorothalonil, Rovral, Dithane M-45</td>
<td></td>
</tr>
</tbody>
</table>

#### Systemic

| RD<sub>1</sub> | Oxadixyl 1 spray |
| RD<sub>2</sub> | Oxadixyl 2 sprays |
| RD<sub>3</sub> | Oxadixyl 3 sprays |
| RD<sub>4</sub> | Oxadixyl 4 sprays |
| T<sub>1</sub> | Ridomil 1 spray |
| T<sub>2</sub> | Ridomil 2 sprays |
| T<sub>3</sub> | Ridomil 3 sprays |
| T<sub>4</sub> | Ridomil 4 sprays |
| - Tonsin 1 spray |
| - Tonsin 2 sprays |
| - Tonsin 3 sprays |
| - Tonsin 4 sprays |
FIG. 13: COMPARATIVE EFFECT OF SYSTEMIC AND NONSYSTEMIC FUNGICIDES ON THE YIELD OF RAPESEED AND MUSTARD
The yield improvement by non-systemic fungicides was only marginal and not significant in most of the cases. Dithane M-45 had significant yield improvement only during the year 1988-89 but not during the following year.
DISCUSSION

Rape seed and mustard are the second most important sources of edible oil in India after groundnut but their productivity is one of the lowest in the world.

Among the various constraints that limit their productivity, damage caused by white rust \( A. \ candida \), often in association with downy mildew \( P. \ parasitica \), is very serious. Cultivars of \( B. \ juncea \) and \( B. \ campestris \), particularly those of the former that are now extensively grown in India are highly susceptible to these diseases. Screening of germplasm against white rust presents difficulties due to lack of knowledge on its race situation since the existence of several biologic races of this pathogen has already been reported (Pound and Williams, 1963; Verma et al.: 1975, Delwiche and Williams, 1977).

Further, both \( A. \ candida \) and \( P. \ parasitica \), being obligate parasites cannot be cultured and maintained on synthetic media for \textit{in vitro} studies, nor they can be effectively controlled through prophylactic spray with conventional non-systemic chemicals. Knowledge of the biochemical changes that take place in host plant following white rust infection is also not adequate. Experiments carried out on these aspects and the results obtained are discussed here under.

The ubiquity of \( A. \ candida \) can be appreciated if one refers to Biga's (1955) compilation of the 241 host species or to the U.S. Department of Agriculture Plant Disease Handbook (Anonymous, 1960), where 44 host species in 21 genera of crucifers is listed. The organism
is an obligate parasite which exhibits both morphologic and biologic specialization.

Pound and Williams (1963) reviewed the early literature on biological specialization in *A. candida* and described 6 races: race 1 on *R. sativus*, race 2 on *B. juncea*, race 3 on *Armoracia rusticana* (Gaertn., Mey. and Scherb) (horseradish), race 4 on *Capsella bursa pastoris*, race 5 on *Sisymbrium officinale* (L.) Scop. (hedgemustard), and race 6 on *Rorippa islandica* (Oeder). Later, race 7 was reported on *B. campestris* (Verma *et al.*, 1975) and race 8 on *B. nigra* (L.) Koch (black mustard) (DeWiche and Williams, 1977).

Each of the isolates collected initially from a particular host species represented a distinct biologic form. Although other species appeared qualitatively differentials for a given isolate, the original host was a much clearer differential quantitatively for the isolate.

*Albugo candida* has been known to infect several host species in India but no study has so far been conducted in order to determine the race situation of the fungus prevalent in the country. Knowledge on the race flora prevalent in a particular region is also an essential pre-requisite for any breeding programme for disease resistance. Keeping this in view, isolates of *A. candida* from three commonly cultivated species of Brassica were collected and cross inoculated on a set of host differentials in order to determine their race identity.

A study of the reaction of differential host species to *B. juncea* isolate of *A. candida* (Table 3) reveals severe disease
intensity on both cotyledonary and mature leaves of the two cvs. Pusa bold and Varuna of *B. juncea*. The percentage of plants infected was also very high, 90 per cent of the plants being infected in both cases.

This isolate also showed similar reaction on *B. nigra*. However, on *B. campestris* cultivars which is the differential host species for race-7, it showed reaction in only one out of the six inoculated. The rest of the differentials did not take up any infection.

These results show that the *B. juncea* isolate of *A. candida* is similar to race 2 (Pound and Williams, 1963) of the pathogen as it is highly virulent on both *B. juncea* and *B. nigra* cultivars but not on those of *B. campestris*, except the cultivar PT-303.

In case of the *B. campestris* isolate of *A. candida*, moderate reaction was observed on the two *B. juncea* cultivars viz., cvs. Pusa bold and Varuna. Interestingly, it also gave low reaction on the *B. juncea* cultivar Domo, which did not show any response to the *B. juncea* isolate of the pathogen.

The reaction on *B. nigra* too was moderate. However, the reaction of this isolate on the cultivars of *B. campestris* which is its own differential host species, is very significant. Out of the six cultivars of *B. campestris* inoculated, only four took up infection. Of these, two showed moderate reaction while the other two gave low reaction.
Although the two *B. juncea* cultivars, one *B. nigra* cultivar and two *B. campestris* cultivars showed moderate reaction of this *B. campestris* isolate of *A. candida*, an appraisal of the data shows that in case of the cvs. Pusa bold and Varuna of *B. juncea* and the *B. nigra* cultivars, the percentage of infected plants was 90, 90.9 and 50 respectively, while in case of the *B. campestris* cultivars the percentage of plants infected was very low, being 18.7 per cent and 15 per cent in case of PT 303 and T-9 (Toria), respectively.

In case of *B. juncea* and *B. nigra* both cotyledonary as well as mature leaves were infected but in case of the *B. campestris* cultivars infection on these two types of leaves was observed in only one case.

Thus, it may be concluded that the present *B. campestris* isolate of *A. candida* belongs to the same race as that of the first isolate, since it produces higher disease severity on the cultivars of both *B. juncea* and *B. nigra*, than on those of *B. campestris*. *B. nigra* isolate, on the other hand, not only infected cultivars of both *B. juncea* and *B. nigra* with equal disease severity but the percentage of infected plants in the two cases was also similar. Infection of *B. juncea* cultivars by *B. nigra* isolate of *A. candida* is expected because the former carries a genome of the latter. However, the mere fact that *B. nigra* isolate also infects cultivars of both *B. campestris* and *B. napus*, though producing low to moderate disease severity, it can not be classified as a separate race.

From the foregoing account it is clear that all the three
isolates, though showed some variation in their reaction, readily cross infected the three host species and produced consistently high disease severity on cultivars of *B. juncea*, the host differential of race-2.

Establishment of a dual culture consisting of an obligate parasite or a near obligate fungal biotroph and its host through the use of tissue culture technique under sterile conditions provides the opportunities to maintain their axenic culture besides studies on the interaction between the two organisms (Morel, 1948). In a series of pioneering experiments, Morel successfully inoculated callus tissue cultures of *Vitis vinifera* (vine) with aseptic zoospores of the downy mildew fungus *Plasmopara viticola* and was able to maintain the resulting dual cultures for extended periods by subculture and re-inoculation.

Since that time, several other dual cultures of fungal biotrophs and their hosts have been established (Ingram, 1973), although the number of species involved is remarkably small and few have been investigated physiologically.

For the establishment of a dual culture of *A. candida* and its host, it was considered essential to first obtain growth of the host tissue in tissue culture medium under *in vitro* condition. In Murashige and Skoog (1962) basal medium, the concentration of hormones was varied to obtain callusing, shooting and rooting. Leaves, stem and inflorescence axis showed growth in the medium but the growth response of the leaf explant was faster than those obtained from the stem and inflorescence axis.
Subculturing of the callus or the plantlet into fresh medium was essential after a certain period of time to avoid their browning and death due to depletion of nutrients in the agar medium. If subcultured into fresh medium at a proper time, renewed growth of the callus or plantlet took place. It was also observed that the time interval for subculturing of the callus could be increased by lowering the temperature at which it is incubated. Due to low temperature, the growth of the callus as well as the rate of its nutrient uptake becomes slow, enabling the callus to remain viable on the same medium for a longer period of time (Table 6).

Interestingly, it was also observed that the callus tissue during course of its development often shows rooting and shooting on the same medium indicating that it is the characteristic feature of the cv. Pusa bold of B. juncea. The roots produced penetrated deep into the medium and thus served the purpose of absorption and transportation of nutrients for the growing callus or plantlet. They were also produced from the portion of the explant which was outside the medium but the rootlets formed soon penetrated the medium (Plates 3 and 4).

To obtain dual culture of an obligate fungal parasite and its host, several methods were used by earlier workers. For example, Morel (1944, 1948) placed surface sterilized vine leaves infected with Plasmopara viticola and allowed the fungus to sporulate on leaves under aseptic conditions. He used these spores to inoculate the vine callus culture. Again, to obtain dual culture, contamination free inoculum was obtained by infecting detached cotyledonary leaves under sterile
conditions in case of *Peronospora farinosa* (Ingram and Joachim, 1971), *P. parasitica* (Ingram, 1969b) and *Bremia lactucae* (Mason, 1973).

In the present study, when sporangia from a surface sterilized unruptured pustule of *A. candida* was used as inoculum, the callus did not get infected and showed normal growth. This might be due to the fact that sporangia from unruptured pustules were immature and did not germinate to cause infection. Eberhardt (1904), Raabe and Pound (1952), Mishra and Chona (1963), Endo and Linn (1960) and Edie and Ho (1970) working on different species of *Albugo* also concluded that age of sori from which the conidia are taken is definitely an important factor in their germination.

Verma (1987) showed that sporangia harvested from mature freshly ruptured pustule showed highest germinability (viz. 30.93%) and produced highest disease intensity. Hence, attempts were made to obtain dual culture by inoculating healthy Brassica callus with sporangial inoculum obtained from freshly ruptured pustule but this invariably caused contamination of all the inoculated callus cultures with saprophytic fungi which later proliferated on to the surrounding agar medium. This result establishes the fact that, ruptured pustules of *A. candida* get easily invaded by secondary saprophytic organisms and the inoculum obtained from such pustules contaminate the callus preventing its further growth.

Thus, it is difficult to establish a dual culture when sporangial inoculum of white rust obtained from a naturally infected plant is used to inoculate a healthy host callus.
All attempts to establish dual culture also ended in failure when rust infected cotyledonary leaf explants, explant with either ruptured or young unruptured rust pustule and also leaf explant from the margin of rust pustule, were used as explant (Expts. 4.2.1 and Table 7).

Leaf explants which yielded saprophytic fungal growth gradually turned brown and died. Some of the inoculated cotyledonary leaf explants developed into normal healthy callus probably because of their escape from rust infection and were, thus, actually healthy. On the other hand, in case of explants with young unruptured pustule, the pustule matured and released its spore mass into the media tube but the surrounding host tissue gradually turned yellow and died (Plates 8, 9 and 10). It is the localized nature of the pathogen in leaf tissue that probably prevented its migration and proliferation into the callus that developed from the distal healthy part of the explant. Similarly, explant obtained from the margin of rust pustule died either before or after limited callusing and formation of a few rust pustules.

White rust causes both local and systemic infection. In case of local infection, rust pustules are formed on leaves and other aerial parts of the plant while systemic infection results in swelling and distortion of inflorescence. It is, however, not known why the pathogen does not become systemic in leaves of rape seed and mustard as in the case of other crucifers. However, as the rust pustule grows the surrounding leaf tissues gradually turn necrotic and die. This death of cells around the rust pustule might possibly be the reason for the failure of infected leaf explant in developing into a white rust infected callus.
Because of these difficulties in infecting callus tissues with fungal spores, early workers attempted to initiate dual cultures by incubating pieces of surface sterilized, systemically infected host tissue on suitable culture medium.

This technique was first used by Hotson and Cutter (1951) with Gymnosporangium juniperi-virginianae which causes telial galls on Juniperus virginiana. The method has since been used with other host and parasite combinations, notably Uromyces ari triphylli and Arisaema triphyllium (Cutter, 1960), Plasmodiophora brassicae and Brassica sps. (Ingram, 1969a), Cronartium ribicola and Pinus monticola (Harvey and Grasham, 1970). Ingram (1969a) initiated infected callus cultures from Plasmodiophora brassicae clubs from 4 to 6 weeks old plants. Sterilized systemically infected clubs were grown on a suitable tissue culture medium and the pathogen could be maintained in complete balance with the host callus.

Using systemically infected tissue, Hardev Singh (1963) was the first to establish Albugo ipomoeae - pandurate in association with Ipomoea pentaphylla. Hypertrophied stems, petioles and flower galls due to systemic infection of A. ipomoeae pandurate, were used. Small sterilized portions of the galls were inoculated on the appropriate tissue culture medium. The gall tissue proliferated to form callus which was maintained for over a year by subculturing at intervals of less than two months. In the present study, when different organs from the hypertrophied inflorescence were aseptically transferred into the callusing media tubes, they responded differently.
Anthers showed no response in the callusing medium while ovary after showing signs of callusing also died. Sepals, petals and flower buds proved unsuitable. But when thickened apical leaf pieces with or without white rust pustule were transferred into the medium, their response was interesting. The former showed limited or low (++) callusing while the latter showed enlargement and formed callus which was rated as good (+++).

Similar results were obtained when thickened but systemically infected inflorescence axis (peduncle) with and without a rust pustule were introduced into the callusing medium. Explants from thickened inflorescence axis (peduncle) without rust pustule showed much more rapid callusing rated excellent (++++), as compared to the one with rust pustule in which case the callusing was rated as good (+++).

While it is difficult to offer any satisfactory explanation for this type of differential callusing response of systemically infected leaf or peduncle explants with and without rust pustule, it can reasonably be assumed that explant with rust pustule might contain fungal metabolite(s) due to localized infection which interfere in callus formation.

However, the response of the hypertrophied inflorescence axis of cv. Pusa bold used as explant was much more than any other explant used in the present study and its callusing could be easily rated as excellent (++++). The growth response exhibited by its healthy counterpart was very low in comparison (Plates 14 and 15, Table 10 and Fig.2).

Microscopic examination of the rapidly proliferating callus
revealed the presence of abundant coenocytic intercellular mycelium which produced numerous globular haustoria within the callus tissue cells. Such fungal mycelium was observed in every callus developed from an hypertrophied inflorescence axis.

Hardev Singh (1963), during his studies on dual culture of *A. ipomoeae-pandurate* on *Ipomoea pentaphylla*, also observed coenocytic mycelium freely proliferating in the intercellular spaces in the callus and producing small round haustoria in abundance inside the host cells. The infected callus obtained by him could be visually distinguished from a healthy one which was not the case in the present experiment.

In the present study, the dual culture obtained showed sustained growth and proliferation, which could be maintained by timely subculture into fresh callusing medium tubes. In this manner the infected callus could also be multiplied rapidly.

After a few subcultures, chains of sporangia having disjunctor cells between them were observed in the callus. These were typically like those of *A. candida*. Hardev Singh (1963) however, reported white globules on the *A. ipomoeae-pandurate* infected callus of *Ipomoea pentaphylla* which showed long chains of conidia coming out of the callus surface.

It has been reported by previous workers that the mycelium of *A. candida* grows and ramifies, and when a certain stage of maturity is reached, produces sporangiophores which bear sporangia (Alexopoulos
The observation that sporangia are formed in the callus after a few subcultures confirms the reports that sporangia are formed after a certain stage of maturity of the fungus.

Formation of oospores within the callus cultures has also been reported by Hurdev Singh (1963) but he did not mention the importance of temperature in the formation of oospores of Albugo. During the present study, it was observed that increase in the temperature of incubation favoured the formation of oospores within the callus.

The above experiment establishes without doubt that, a dual culture of *A. candida* and cv. Pusa bold of *B. juncea* can be established *in vitro* using the hypertrophied inflorescence axis as explant. The fungus ramified within the host callus and could be maintained in equilibrium with it. Also the infected callus showed pronounced growth as compared to its healthy counterpart which confirms the observation made by Verma (1987) that *A. candida* is the principal pathogen in bringing about floral hypertrophy in rapeseed and mustard. The role of *P. parasitica* in causing the hypertrophy in Brassicas will be clarified while discussing the next experiment.

For the maintenance of *A. candida* infected callus tissue, subculturin of the callus into a fresh medium proved to be the most appropriate method. Attempts to infect a healthy callus of cv. Pusa bold by placing a systemically infected tissue in juxtaposition with it was unsuccessful although such a method was successfully used by Griffin and Coley Smith (1968) in case of *Pseudoperonospora humuli* - hopstem and by Tiwari and Arya (1969) in case of *Sclerospora graminicola*.
Pearl millet system.

To obtain dual culture of *P. parasitica* and its host, four types of explants—viz., (i) *P. parasitica* infected leaf, (ii) Leaf pieces from the margin of *P. parasitica* lesion, (iii) Leaf pieces with young unruptured white rust pustule and (iv) *A. candida* induced hypertrophied inflorescence axis having growth of *P. parasitica* on their surface, were introduced into media tubes and incubated at 18±2°C.

It was observed that transference of infected leaf pieces into media tubes results in their cent per cent contamination and this may be due to their selection at an advanced stage of infection by the downy mildew fungus which favoured the invasion of other contaminants in their tissues. On the other hand, when leaf pieces from the margin of downy mildew lesion were taken most of them died after their transference into media tubes indicating that Peronospora has a killing effect on host tissue and thus prevents them from proliferating and growing into a callus.

Leaf pieces bearing young unruptured white rust pustules yielded both pure culture of *P. parasitica* and a mixed growth, consisting of the downy mildew fungus and a sterile hyphae of unknown identity. Peronospora grew out from the area surrounding the white rust pustule.

Bains and Jhooty (1984) also reported that in nature, *P. parasitica* appeared in and/or around the white rust pustules of mustard plants. These results only confirm the view that infection with
A. candida and/or P. parasitica makes the host tissue very vulnerable for the entry of other organisms including P. parasitica. Interestingly, it was also noticed that when young white rust pustule is invaded by P. parasitica the former never matures and ruptures in vitro, a phenomenon which is observed when A. candida is not in association with Peronospora.

Pure culture of the downy mildew fungus could also be obtained when A. candida induced hypertrophied inflorescence axis having incipient growth of P. parasitica on its surface was used as explant.

However, explants showing the growth of P. parasitica exhibited rapid senescence and progressively turned brown. The fungus continued to sporulate until the eventual browning and death of the host callus. Thus, the Peronospora infected callus could not be maintained by subculturing into fresh medium since the infected tissue aged rapidly in contrast to A. candida infected callus which could be readily subcultured.

However, when a portion of P. parasitica infected callus was kept in juxtaposition with a healthy or A. candida infected callus and incubated, the downy mildew pathogen migrated into the new callus tissue giving rise to typical Peronospora colonies. In contrast, such a method did not lead to migration of the white rust fungus to a healthy callus.

Successful growth of dual culture is dependent upon the establishment of a balanced relationship between the component organisms.
comparable with that existing in nature. This is not always possible (Brian, 1967), although in some instances the natural equilibrium between host and parasite may not only be retained in culture but enhanced. It is perhaps not surprising that most of the combinations which can so far be placed in this category involve fungi which have a systemic growth habit and cause morphological disturbance in the host plant e.g. Sclerospora graminicola (Tiwari and Arya, 1969). Peronospora farinosa (Ingram and Joachim, 1971) Gymnosporangium juniperus – virginianae (Hotson and Cutter, 1951). Cronartium ribicola (Harvey and Grasham, 1971) and Plasmodiophora brassicae (Ingram, 1969a).

The ability to live in balance with host tissue cultures differs with different host fungus associations. In case of the dual culture of A. candida and its host, there is a balanced relationship between the two. So much so, that its repeated subculturing did not result in the loss of A. candida indicating that the fungus keeps pace with the growth of the callus.

In some cases a reverse situation has also been reported by earlier workers where a vigorous host tissue outgrows the fungus. This was sometimes the case with dual culture of sugarbeet with P. farinosa (Ingram and Joachim, 1971). Hardev Singh (1963) also reported that some of the explants of Ipomoea pentaphylla became free of A. ipomoeae-panduratae after repeated subculturing.

It is of interest to record here that A. candida infected callus tissue of cv. Pusa bold proliferated more rapidly than its healthy counterpart, a phenomenon very similar to the behaviour of the fungus
when it systemically infects the inflorescence of Brassica under natural conditions. The infected callus also retained its ability to grow faster than its healthy counterpart even after subculture. Such a behaviour was also observed in Brassica callus tissue infected with Plasmodiophora brassicae which proliferated more rapidly than the healthy callus (Ingram, 1969a and b; Williams et al., 1969; Dekhuijzen and Overleem, 1971).

On the other hand, in case of P. parasitica infected callus of cv. Pusa bold, a balanced relationship between the two was not observed and rapid browning and death of the host callus took place. Hence, the fungus could not be maintained by subculturing the infected callus. This confirms the observations reported by Ingram (1969b) while growing P. parasitica in vitro on Brassica callus. He too found that the callus tissues of Brassica species are killed within 7 to 14 days of infection with P. parasitica.

Butler (1918) observed frequent coexistence of Cystopus candida (A. candida) and P. parasitica on cruciferous hosts and said that though it is not easy to separate their effect, Perenospora produced more deformities in the stem, Cystopus in the flowers. In inflorescence infested with Perenospora, flowers are not swollen except the ovary.

Verma (1987), however, stated that A. candida is the principal pathogen in bringing about floral hypertrophy in rape seed and mustard while P. parasitica plays a very minor role, if at all, in this respect.
Results obtained during the present investigation confirm the view that systemic infection by *A. candida* leads to enlargement (hypertrophy) of the host tissue while infection with the downy mildew fungus leads to its death. It is *A. candida*, which first induces hypertrophy and this makes the host tissue prone to the attack of *Peronospora*.

Investigation was also taken up to determine the extent of changes that may occur in the biochemical constituents of white rust infected inflorescence of cv. Pusa bold with the progress of malformation process. The three stages of malformation that were chosen for analysis were a) Initial stage - tissue swelling incipient, b) Middle stage - tissue swelling pronounced, c) Final stage - distortion and malformation complete.

Analysis of healthy and malformed inflorescence showed the existence of a direct relationship between the concentration of sugars and the progress of the disease.

The concentration of both reducing and non-reducing sugars gradually increased as the hypertrophy became more pronounced, the increase being more between the initial and middle stage. Maheshwari et al. (1985) also noted higher concentration of carbohydrates in hypertrophied inflorescence axis of *B. juncea* due to *A. candida* and attributed the increase to be due to the higher activities of the pathogen (Maheshwari and Chaturvedi, 1976) or to both host and parasite activity (Chadha and Srivastava, 1971). However, this increased accumulation of reducing and non-reducing sugars in inflorescence, whose cells are
under rapid hypertrophy and hyperplasia may also be attributed to their higher synthesis by the host due to increased photosynthetic activity since all the floral organs turn green.

Similarly, a gradual increase in the free amino acid content of the inflorescence from the initial to the final stage of malformation was observed. Increase in free amino acid content was also noted by Verma (1987) in inflorescence of Brassica species as a result of infection with A. candida and by Prasada (1983) in coriander infected with stem gall disease. Such increase in the concentration of free amino acids is attributed to the breakdown of host proteins by the pathogen and/or activation of enzymes responsible for amino acid and amine synthesis.

Phenols, their oxidation products and derivatives are known to confer resistance in plants against many fungal pathogens. During the present study it was observed that the total phenol content declined with the progress of white rust infection. The decline in the concentration was more or less gradual, although the decline was more prominent between the initial and middle stages of malformation.

Dhingra et al., 1982, estimated the changes in total phenolics in floral parts of B. campestris infected with A. candida. They too observed a significant decrease in total phenolics in all the infected floral parts and floral axis. Verma (1987) also reported a decrease in total phenols in the inflorescence of B. juncea due to infection with white rust.

This reduction in phenol content of Brassica species following infection with white rust might be due to their oxidation into
quinones by phenol oxidase enzyme of the pathogen (Tomiyana, 1963) or due to its polymerization into nontoxic compounds like melanins (Kuc, 1963).

Studies on the crude protein content also showed that white rust infection causes a decrease in crude protein content of inflorescence and maximum decrease occurred between the initial and middle stages of malformation. Similar results were obtained by Dhingra et al. (1982) when analysing the floral parts of *B. campestris* infected by *A. candida*. Verma (1987) also confirmed that a decrease in proteins take place in the inflorescence due to white rust infection. In contrast, Maheshwari et al. (1985) conducting histochemical studies on *A. candida* induced hypertrophied inflorescence axis of *B. juncea*, found an increase in the concentration of proteins. However, this reduction in protein content and increase in amino acids concentration might be due to break down of the former by the pathogen. This needs confirmation.

The infected inflorescence showed increase in moisture content upto the middle stage of malformation and thereafter, the moisture content decreased as it reached the final stage. Increase in moisture content of malformed inflorescence of Brassica due to *A. candida* has also been noted by Dhingra et al. (1982) and Verma (1987). Increase in moisture content during the early phase of malformation may be due to increased permeability of hyperplastic inflorescence cells, the latter being the result of higher accumulation of indole acetic acid as suggested by Waziri et al. (1974). On the other hand, the decrease in moisture content during the later stage may be attributed to its loss during tissue maturation and drying.
Analysis of the *A. candida* infected inflorescence for nitrogen, phosphorus and potassium content at the three stages of malformation showed a gradual decline in nitrogen content from the initial to the final stage while the concentration of the other two elements in the malformed tissue decreased up to the midstage and then slightly increased at the final stage.

Similarly, the concentration of copper, zinc, iron and manganese decreased with the progress of malformation, the decrease of the first two being more up to the midstage than at the final stage while iron and manganese showed more decrease at the final stage. These results provide no indication about their role in malformation process except that the disturbed physiological condition of the diseased inflorescence is likely to have profound influence upon their uptake and utilization.

To have a proper understanding of the biochemical differences between leaves and inflorescence of a white rust susceptible cultivar before and after infection with *A. candida*, and also to compare the differences between a susceptible and resistant cultivar of *B. juncea* namely, cv. Pusa bold and Bio YSR, a number of analysis were undertaken.

Many workers are of the opinion that there may be a direct relationship between the sugar content of plants and their susceptibility to various diseases. The sugar content of leaves and inflorescence of the resistant cultivar was found to be higher than that in their healthy counterparts at the susceptible cultivar. This is in agreement with the results reported by Verma (1987). Similar differences in sugar content
has also been reported in wheat against stem rust by Lyles et al. (1959) and Krog et al. (1961), in groundnut resistant to Cercosporidium personatum (Swamy, 1964) and in maize against Cochliobolus heterostrophus. (Agarwal, 1978).

On the contrary, Singh et al. (1987) reported higher reducing sugar content in most susceptible varieties of potato to Alternaria alternata infection which decreased with the increase in resistance of potato varieties.

Thus, white rust falls under the category of low sugar disease of Horsefall and Dimond (1957) since the Brassica cultivar with higher sugar content is resistant to it.

However, after infection with A. candida, there was a drop in sugar content in the foliage of the susceptible cultivar and conversely, an increase in sugar content took place in its floral parts. Thus, the changes in the metabolic process brought about by the pathogen in the leaves where it is localised is very different from that in the inflorescence, where it is systemic in nature.

A drop in the sugar content in the leaf tissue following infection has also been reported by Verma (1987). However, she reported post-infectional drop in the inflorescence too, which is contrary to the present observation.

The reduction in sugar content in the infected leaves may be due to utilization of sugars by the pathogen, decrease in photosynthetic activity and/or increase in respiration rate of the infected leaves and
marshelling of a portion of sugars for the biosynthesis of polyphenols.

Recently, Lukose et al. (1990) reported reduction of total sugars in rust and downy mildew infected bajra leaves. They also reported higher levels of sugar in resistant cultivars as compared to susceptible ones. Narula and Mehrotra (1990) also noted a marked reduction in the level of reducing and non-reducing sugars during pathogenesis in Phytophthora infected leaves of colocasia.

Interestingly, in both resistant and susceptible cultivars, the sugar concentration of inflorescence was much more than that of their leaves. This is in confirmation of earlier results obtained in case of malformed inflorescence.

Analysis of the two Brassica cultivars showed higher concentration of free amino acids in healthy leaves and inflorescence of the susceptible cultivar as compared to those of the resistant cultivar. Apet and Suryawanshi, (1990) also observed that the amino acid content of the groundnut variety susceptible to Puccinia arachidi is higher as compared to that in resistant cultivars.

With a view to ascertain the biochemical nature of resistance, Taneja et al. (1990) estimated the free amino acids in Myrothecium leaf spot resistant and susceptible varieties of cotton. They reported the presence of six amino acids in healthy leaves of susceptible cultivars of cotton but their absence in infected leaves clearly indicated that these amino acids were efficiently utilized by the fungus during the process of pathogenesis. They further noted the absence of six amino
acids and the presence of leucine and tryptophan in the resistant varieties and suggested that these may be responsible for imparting resistance against the pathogen.

In the present study, an increase in the concentration of free amino acids was noted in both leaves and inflorescence following infection with white rust. The resistant cultivar, on the other hand, showed lower amino acid concentration in both leaves and inflorescence as compared to the susceptible cultivar. These results are in conformity with that obtained earlier in case of inflorescence undergoing malformation.

Verma (1987), however, found no indication of the involvement of amino acid content of plants with their resistant/susceptible reaction to white rust infection. However, the possible reasons for increase in amino acids following infection has been discussed in the earlier experiment.

The role of phenols in disease resistance of plants has been studied by numerous workers. When comparing the levels of phenols in white rust susceptible and resistant cultivars of Brassica, a higher concentration of this compound was observed in the resistant one. This is in accordance with the results reported by Verma (1987) in case of white rust disease of Brassica.

In some instances phenolic content has been found to increase after infection (Sridhar and Ou, 1974, and Sharma et al., 1983). Narula
and Mehrotra (1990) also reported more phenols in the diseased leaf tissues of colocasia infected with *Phytophthora colocasiae*. Similar results were obtained from the work of Lukose et al. (1990), who observed increase in total phenols in bajra leaves infected with blast (*Pyricularia penniseti*), leaf spot (*Helminthosporium australiense*), rust (*Puccinia penniseti*) and downy mildew (*Sclerospora graminicola*).

However, during the present experiments, a net decrease in phenol content was recorded in the leaves and inflorescence of susceptible cultivar after infection with white rust. Verma (1987) also reported a decrease in total phenol content due to infection of Brassica cultivars with white rust. Decrease in phenol content due to infection by a pathogen was also reported by Sindha et al. (1978) and, Rao and Dua (1978) while working with sunflower leaves infected by *P. helianthi* and with Amaranthus leaves infected by *A. bilti*, respectively.

Similar decrease in phenol content was also observed in the earlier experiment carried out with infected inflorescence undergoing malformation and the possible cause of such a decline has been discussed.

As in the above case, crude protein content of both leaves and inflorescence of the resistant cultivar was lower than that in their susceptible counterpart. After infection with white rust, their concentration in both leaves and inflorescence of the susceptible cultivar decreased.

The changes taking place in the inflorescence due to hypertrophy formation has already been discussed.
In case of leaves, Verma (1987) too reported similar observations while working with *A. candida* infected cultivars of *Brassica*.

On the other hand, Sindha *et al.* (1978) reported a decline in the concentration of soluble protein in sunflower leaves susceptible to *P. helianthi* but its increase in resistant leaves, 10 days after inoculation. Further study is required to confirm the results with respect to white rust reaction on *Brassica* cultivar.

Moisture content in both susceptible and resistant cultivar was the same and this cannot be implicated in their susceptible or resistant reaction to white rust. However, the moisture content of inflorescence increased due to infection, as has been discussed earlier but the reverse took place in the leaves. The reduction in water content in the leaves may be attributed to increase in transpiration rate due to infection and death and necrosis of the leaf tissue.

Analysis for mineral content of the healthy and white rust infected leaf tissues of the susceptible cv. Pusa bold showed a decrease due to infection by the pathogen. This may be the result of their reduced uptake by the diseased plant as suggested earlier. Also the concentration of all the minerals except zinc was higher in the leaf and inflorescence of the resistant cv. Bio YSR as compared to the susceptible cv. Pusa bold. Further, investigations are required to understand the role of minerals, in the susceptible/resistant reaction of the plant to white rust.

All the systemic and non systemic fungicides tested for their
eficacy against Alternaria blight and white rust of mustard brought about a reduction in disease intensity. Significant reduction in disease intensity was observed when Ridomil and Dithane M-45 were used, the former being better than the latter.

Besides toxicity, persistence is another criterion that determines the efficacy of a fungicide. Degradation, which determines persistence of fungicides on the host surface, is the result of interaction between the various factors of the environment and the substances that are present on the leaf surface.

The higher efficacy of Ridomil over that of Dithane M-45 in reducing the disease intensity is also reflected in seed yield of plants. Application of this systemic fungicide resulted in much higher seed yield than when the non-systemic fungicide, Dithane M-45 was applied.

Out of the three non-systemic fungicides used Dithane M-45 proved to be the best followed by Rovral and Chlorothalonil. Among the systemic fungicides used, Ridomil proved to be the best. It was closely followed by Oxadixyl while Topsin was the least effective for disease control and resultant increase in seed yield.

Increasing the number of sprays of the systemic fungicides progressively and significantly reduced the incidence of both the diseases. Superiority of Ridomil over the other two fungicides was evident as significant results were obtained even with two sprays while in case of others significant decrease in disease intensity could be achieved with at least three sprays.
SUMMARY

Among the various constraints that limit the productivity of rapeseed and mustard, damage caused by white rust is very serious. The present study yielded information of both basic and applied value on some of the unresolved but most important aspects of the pathogen and the disease.

Screening of germplasm for evolving white rust resistant cultivars presents difficulties due to lack of knowledge regarding the race situation in India. Therefore three isolates of the pathogen parasitizing three commonly cultivated species of Brassica viz., *B. juncea* (mustard), *B. campestris* (yellow sarson) and *B. nigra* (black sarson) were tested on a set of differentials to ascertain their race identity.

*B. juncea* isolate of *A. candida* used in the present experiment was highly virulent on the cultivars of both *B. juncea* and *B. nigra* but not on those of *B. campestris* except the cultivars PT 303 indicating that it is similar to race 2 of the pathogen.

The *B. campestris* isolate of *A. candida* showed high degree of infection on both *B. juncea* and *B. nigra* cultivars like that shown by the *B. juncea* isolate of the pathogen but it differed from the latter isolate in its pathogenicity on *B. campestris* where it induced low to moderate reaction in only four cultivars out of the six inoculated.

Result of experiment with *B. nigra* isolate also showed highest severity of the disease on the two *B. juncea* cvs. Pusa bold and
Varuna besides B. nigra. The results were similar to those obtained in the previous two inoculation experiments. From the foregoing account it is clear that all the three isolates, though showed some variation in their reaction, readily cross infected the three host species and produced consistently high disease severity on cultivars of B. juncea, the host differential of race 2.

It can thus be concluded that the three isolates of A. candida behaved more or less similarly and can be classified as race 2 of the pathogen as reported by Pound and Williams, 1963).

Experiments were taken up to establish dual culture of the obligate parasites Albugo candida and Peronospora parasitica on their common host, B. juncea cv. Pusa bold.

Callus cultures as well as plantlets of cv. Pusa bold were produced on the callusing medium using leaves, stem or inflorescence axis of the plant as explant. Better results were obtained when leaves were used as explants.

Subculturing of the active callus tissue into fresh callusing medium resulted in cent per cent success in obtaining fresh callus growth but when done after the onset of browning no regeneration took place. The optimum time interval required for subculturing of a callus for its incubation temperature. The period of subculturing of the callus could be increased by lowering the temperature of its incubation.

Root and shoot formation were observed while the callus was growing in the callusing media itself. Rooting was observed as early
as 7 days after the explants were introduced into the callusing medium. Shoot formation was, however, observed on 15 to 20 day old callus. Shoot bud differentiation took place from the peripheral cells.

Various methods were tried for the establishment of dual culture of *A. candida* and its host. Inoculation of healthy callus of cv. Pusa bold with sporangia obtained from ruptured white rust pustules yielded only various fungi as secondary invaders (contaminants) but not *A. candida* but when sporangial inoculum was taken from an unruptured pustule to inoculate the callus no visible change in the callus could be noticed. The callus did not become infected and showed normal growth.

All attempts to establish dual culture also ended in failure when rust infected cotyledonary leaves as well as those with either ruptured or young unruptured rust pustule and also leaf pieces from the margin of rust pustule were used as explant.

In case of explants with young unruptured pustule, the pustule matured and released its spore mass into the media tube but the surrounding host tissue gradually turned yellow and died.

Attempts were made during the present study to induce dual culture by incubating different parts of *A. candida* induced hypertrophied inflorescence. Anthers when used as explant showed no response in the callusing medium while ovary, after showing signs of callusing, also died. Sepals, petals and flower buds proved unsuitable. But when thickened apical leaves with and without white rust pustule were transferred into the medium, the former showed limited or low (++) callusing while latter showed enlargement and the callus formed was rated as good (+++).
When hypertrophied inflorescence axis with and without a rust pustule were introduced into the callusing medium, those without pustule showed much more rapid callusing rated excellent (++++) as compared to the one with rust pustule in which case the callusing was rated as good (+++).

Although the callusing of the hypertrophied inflorescence axis of cv. Pusa bold could easily be rated as excellent, the growth response exhibited by its healthy counterpart was very low in comparison. Proliferation of the cells at the cut ends of the explant started much earlier and their growth rate was much faster as compared to cell proliferation and growth rate exhibited by healthy explants. However a callus derived from such explants never gave rise to plantlets or formed roots as in generally observed in case of callus derived from healthy explants of cv. Pusa bold.

Microscopic examination of the rapidly proliferating callus revealed the presence of coenocytic intercellular mycelium in abundance which produced numerous globular haustoria within the callus tissue cells.

This vigorously growing callus covered the entire surface of the medium within 20 days of its introduction into the medium, after which the growth slowed down. If retained in the same medium beyond 25 days without subculturing, the callus turned flaccid and brown in colour. Hence two methods were tried for the maintainence of the fungal culture in its host callus.

When an infected callus was kept in juxtaposition with a healthy Brassica callus migration of the fungus did not take place
from an infected callus to a healthy one. However the dual culture could be maintained by subculturing it into fresh callusing medium tubes after 15 to 20 days interval at 18±2°C.

Formation of the asexual stage of the fungus was observed after the callus had been maintained for about three to four months. Microscopic examination showed chains of sporangia within the callus which were typically like those of *A. candida* found in nature. These sporangial chains were formed away from the centre towards the peripheral region but below the surface of the callus mass. The central portion of the callus did not show presence of any sporangia. The average size of the sporangia varied between 20 to 15 μ.

*A. candida* could be induced to produce its asexual or sexual stage in its host callus tissue by varying the temperature of incubation. It was found that callus maintained at 15°C and 20°C contained only fungal mycelia and sporangia but those maintained at 25°C and 30°C showed the presence of both sporangia and oospores, the relative number of the latter being more in those callus that were maintained at 30°C. The oospores were globate, dark brown and highly differentiated having 5 layered cell wall. The epispore was thick having very prominent ridges. These oospores were 45 to 55μ in size and were formed between the cells of the callus tissue, the cells being pushed backwards by the growing oogonium.

To obtain dual culture of *Peronospora parasitica* and its host different types of explants were used. When *P. parasitica* infected leaf pieces were used as explant it resulted in cent per cent
contamination. Also when leaf explants were derived from the margin of downy mildew lesion, all of them died without showing any growth response.

On the other hand leaf explants bearing young unnuptured white rust pustules often yielded both pure culture of *P. parasitica* and a mixed growth consisting of the downy mildew fungus and sterile hyphae of unknown identity.

It was noticed that when young white rust pustule was invaded by *P. parasitica*, the former never matured or ruptured *in vitro* a phenomenon which was observed when *A. candida* was not in association with *Peronospora*.

Pure culture of the downy mildew fungus could also be obtained when *A. candida* induced hypertrophied inflorescence axis having incipient growth of *P. parasitica* on its surface was used as explant. Explants which yielded *Peronospora* showed aerial sporangioles from their surface within 4-5 days of their introduction into the medium. The growth of the explant was slow but the fungus developed abundantly when incubated at 20\(^\circ\)C.

However, such explants exhibited rapid senescence and progressively turned brown. The fungus continued to sporulate until the eventual browning and death of the host callus. The culture of *P. parasitica* could not be perpetuated by subculturing. It could be however maintained by keeping healthy or *A. candida* infected calluses in juxtaposition with a callus showing sporangial growth of *P. parasitica* on its surface. Fungal hyphae invaded the new tissue after about 7-8 days and
characteristic sporangiophores and sporangia of *P. parasitica* developed on it.

**Biochemical analysis of healthy and A. candida infected inflorescence at the initial, middle and final stage of its malformation** showed a gradual increase in the concentration of both reducing and non reducing sugars and total free amino acids but a decrease in the concentration of total phenols and crude protein with the progress of malformation. The moisture content of infected inflorescence increased upto the middle stage and then declined.

**White rust infected inflorescence also showed a gradual decrease** in its nitrogen content from the initial to final stage of malformation while the concentration of phosphorus and potassium first decreased upto the middle stage and then increased at the final stage. Similarly, the concentration of copper, zinc, iron and manganese decreased with the progress of malformation, the decrease of the first two being more upto the midstage than at the final stage while iron and manganese showed more decrease at the final stage.

**Biochemical differences between leaves and inflorescence of white rust susceptible cv. Pusa bold as well as its difference from the resistant cv. Bio YSR were also examined.** The sugar content of leaves and inflorescence of the resistant cultivar was higher than that in their healthy counterparts of the susceptible cultivar indicating that white rust disease falls under the category of "low sugar" disease of Horsfall and Dimond (1957). The inflorescence contained more sugars than leaves in both the cultivars.
Concentration of free amino acids in healthy leaves and inflorescence of the susceptible cultivar was more as compared to that in the resistant cultivar. Infection with white rust caused an increase in the concentration of free amino acids in leaves and inflorescence.

Total phenol content of leaves and inflorescence of the resistant cultivar was more as compared to that in their healthy counterparts of the susceptible cultivar. Also due to infection with white rust the concentration decreased in both leaves and inflorescence of the susceptible cultivar. In contrast, the crude protein content of both leaves and inflorescence of the resistant cultivar was lower than that in their susceptible counterparts. In the susceptible cultivar a decrease in crude protein content was observed in both leaves and inflorescence due to infection.

Moisture content of leaves and inflorescence of the two cultivars was almost similar, infection with white rust reduced the moisture content of the leaves but increased it in the inflorescence of the susceptible cultivar.

The susceptible cultivar contained higher concentration of nitrogen in its leaves and inflorescence than the resistant cultivar. After infection it concentration increased in leaves but decreased in the inflorescence in the susceptible cultivar. The phosphorus content of the two cultivar was almost similar, but in the susceptible cultivar after infection with white rust, its concentration decreased in both leaves and inflorescence. On the other hand, white rust caused an increase in potassium content in leaves and inflorescence of the susceptible
cultivar. The cultivar was slightly lesser than the susceptible one in both leaves and inflorescence.

Concentration of copper and iron was higher in the resistant cultivar as compared to that in the susceptible cultivar. After infection, the concentration of copper and iron decreased in leaves and inflorescence of the latter.

Manganese content of the resistant cultivar was higher while that of zinc was lower as compared to the susceptible cultivar. White rust infection caused a decrease in the concentration of manganese in leaves and inflorescence besides also lowering the concentration of zinc respectively in the above organs of the susceptible cultivar.

In general application of either systemic or non systemic fungicide considerably reduced the intensity of white rust as well as that of Alternaria blight. Among the various systemic fungicides Ridomil was the most effective followed by Oxadixyl and Topsin. Increasing number of sprays of these fungicides progressively and significantly reduced the incidence of both the diseases. However in case of Ridomil results were significant even with two sprays. In the other two systemic fungicides no difference was observed between three and four sprays thus three sprays was found to be sufficient for an effective control of the two diseases.

Among the three non systemic fungicides, Dithane M-45 decreased the intensity of both the diseases most as compared to Chlorothalonil and Rovral.
Yield improvement due to application of systemic fungicides was markedly more as compared to non-systemic fungicides. Ridomil again gave the best results followed by oxadixyl. Yield improvement by non-systemic fungicides was marginal and not significant in most cases.
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