INTRODUCTION

STUDIES ON SEED HEALTH TESTING: INTERACTIONS AT SEED SOIL INTERFACE.

Early germination of seeds and consequent early emergence of seedlings, is always acceptable to agriculture as seeds and seedlings can escape attack by soil-borne pathogens. Germination depends, to a great extent, upon activity of enzymes which act on storage compounds, and release essential nutrients.

Induction of early germination is a prerequisite for disease escape and therefore demands a careful study of seed enzymes. Physical factors like pH, O₂, water, temperature etc. affecting germination have been studied extensively as revealed through literature on soil-borne pathogens of crop seeds and seed treatments. Enzymes studied in these cases are mainly substrate-based (i.e. seed reserves like starch, protein, fats etc.). Storage substrate reserves are diffuse in case of vegetable seeds. Enzyme profile and enzyme release patterns in these seeds therefore offer a case worth investigation. This study was undertaken to elucidate some of these aspects. Seed-borne fungi and bacteria also secrete enzymes. The normal enzyme release pattern may be altered due to infection or other seed treatments. Investigations along these lines revealed that selective activation of seed enzymes Amylase, pectinase, cellulase, β-Glucosidase, β-Fructofuranosidase, Aminotransferase and proteinase is possible.
Dominant pathogens of *Sorghum vulgare* (Jowar), *Brassica oleracea* (cauliflower), *Capsicum annum* (Chilly), *Daucus Carota* (Carrot), *Lycopersicum, esculentum* (Tomato), *Allium cepa* (Onion), *Solanum melanogena* (Brinjal) were identified after isolation and were used in enzyme studies.

Secretion patterns of enzymes and responses of seeds inoculated by dominant pathogens changed. Thus infection by *Alternaria* changed enzyme release patterns in cauliflower seeds and inoculation of, *Aspergillus* changed enzyme release pattern in sorghum. The treatments also included, in addition to inoculation by fungi and bacteria, soaking or infiltration of seeds by stimulators of seed germination, Inhibitors of seed germination, Vitamins, growth substances and TCA cycle acids. The results indicated alteration in enzyme release/inhibition as well as activation. Attempts have been made to relate seedling growth, seed germination and infection. Phosphate solubilizing enzymes were also located and activation was attempted. Protease and amylase mediated aminoacid and sugar release patterns were studied in detail using seed enzymes. Both amylases and Proteases were found effective in release of glucose and aminoacids from raw flour. Experiments on activation of seed enzymes through extracts from medicinal plants yielded positive results.
REVIEW OF LITERATURE

There is a growing interest in seed Pathology in developing countries, both in terms of seeds for quality and quarantine and in terms of research and teaching (Neergaard, 1975). Educational programmes in seed pathology for developing countries, India included, were started by Danish Government Institute for seed Pathology in 1967. More than a dozen laboratories routinely carry, seed health tests mostly on crop seeds. So far most seed health testing has been concentrated on detection of seed borne fungi and chemical treatments to control seed borne diseases. (Pathak, 1974).

Environmental factors which affect adversely performance of seeds in soil, include, supraoptimal or suboptimal temperatures, drought, flooding, unfavourable light, poor aeration, insects, rodents, birds, infection and proliferation of soil borne and seed borne pathogens, chemical residues and unfavourable structure and composition of soil (Khan, 1977). Restoration of appropriate physiological, chemical, and environmental conditions, would re instate germinability.

The longer a seed remains in the soil, the lesser are its chances of survival. Infection by soil micro organisms, destruction by diseases borne within itself are major factors. Seeds are exposed to changing and often adverse environments with soil for a considerably long period which begins with, sowing and ends with emergence. Numerous efforts have been made to decrease the period between sowing and emergence with the assumption that quick germination, or emergence will spare the seed of
exposure to hostile, environment during imbibition & seedling establishment. Protein synthesis plays an important part in germination in the growth of embryonic axis and in the synthesis of the hydrolytic enzymes and the other cellular machinery used for the mobilization of the reserves, protein synthesis can be shown to occur, with a few hours of the beginning of imbibition before the recommencement of mRNA synthesis.

Interactions between seed and seed borne pathogens reveals mechanisms which decide susceptibility and resistance in majority of cases.

A seed, is an ideal medium for majority of fungi and the nature of substrate typically governs seed mycoflora. Khandal (1982) has found that 34 cotton cultivars typically yielded lipolytic and cellulolytic fungi as dominant ones. Linters provided cellulose, storage reserves provided fats.

Aspergilli were dominant on cotton, oil seeds and also on legumes (Khandal, 1982; Kulkarni, 1979; Muley, 1990).

Cellulase activity in germinating barley has been attributed to contaminating microorganisms and also plants (Ashtord & Gubler 1984). NaOCl treatment inhibited cellulase in sorghum. NaOCl also checked growth of seed borne fungi. Thus here too, cellulases appear to be from contaminating microbes. Cellulases contribute to wall hydrolysis.

THE PLANTS SELECTED:

ALLIUM CEPA (ONION)

Of the mono cotyledonous family, Liliaceae is native from Palestine to India. It was cultivated very early by the ancient
Egyptians, and its use as a food plant can be traced as far back as 3000 B.C. It was also recorded by the Jews before their exile from Israel. The use of the onion was adopted by Europeans from the Eastern Nations and its use persists to this day. There is now no western civilization where it is not used, but in some cases it should properly be considered as a condiment rather than vegetable. The edible part is the bulb, a shortened stem, bearing thick, fleshy colorless lvs. full of food reserves.

The following are major seed borne pathogens of onion. *Alternaria porri*, *A porri*, *F.sp.Porri*, (purple blotch of onion) - *Botrytis Alli*, *Collectotrichum dematium*, *f.sp.Circinans*, (smudge). *Aspergillus niger A.flavus* and *Drechslera australiens*, appear to be the most pathogenic on onion. The maximum damage, is caused by *Aspergillus flavus*. Benlate was found to be the most effective chemical against seed associated fungi.

*Azotobacter chroococcum, Azospirillum braziliens* and / or *Azospirrillum lipoferum* when used in different combination germination rate improved from 85.5% in the untreated lots to 96-5% in the lots treated with A.Chrooccum.

**BRASSICA OLERACEA**

Brassica was introduced into Britain in the seventeenth Century from Cyprus but it appears, to be native to Asia Minor. However Cauliflower was already known in Europe in the sixteenth Century, as is proved by its oldest known description in book by Dutch botanist Dodoens published in 1559. Its edible part is the
solid head, formed by its racemose inflorescence, on a shortened central stem.

Cauliflower grows best in the cool, and moist part of the temperate regions. Large white heads are generally required and a large head of cauliflower may exceed 20 cm, in diameter. It is propagated by means of seeds and is now one of the most important vegetables in all European countries as well as in the English speaking countries overseas.

Following are considered as major seed borne pathogens. *Alternaria brassicae*, *Alternaria brassicicola*, are common in *Brassica oleracea*, and other cruciferous seeds. *Alternaria brassicicola*, (black spots of cabbage) (Noble and Richardson 1968) develops high infection percentages on seeds of cabbage and cauliflower *Brassica oleracea*. *Alternaria rapha*.


These fungi are infectious and are seed transmitted in many cruciferous crops, including cabbage, radish. *Xanthomonas campestris* develops Black rot of crucifers. (Neergaard 1980).

**CAPSICUM ANNUM**

This is one of the important crops grown for the value of its fruits in making spice and condiment. It forms an essential part of the Indian diet, and the fruits are used either dry or raw. It is grown extensively in Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra states and the total area under the crops is over 0.7 million hectares. It is grown on a rainfed crop in most parts of Andhra Pradesh and as an irrigated crop in other
areas. There are several varities of Chilly grown in India and some are non punegent, with large sized fruits that are used mainly as a vegetable.

Following are considered as major seed borne pathogens.

*Alternaria porri* F.Sp.*, solani*.  
*Cercospora capsici*, *Cercospora Capsidicola*, *Colletotrichum*, *Dematium*, *Drechslera Sorokiniana*, *Fusarium equiseti*, *Fusarium monilifor me.*, *Fusarium solani*, *Phoma*, *Phytophthora capsici*, *Xanthomonas vesicatoria*, *Cercospera capsici carthami* and *Cercospera beticola* (Neergaard, 1978.)

Vidhyasekhran and Thyagragajan 1981 stated that *Fusarium oxysporum* reduced seed germination, growth rate and fruit yield. The fungus was frequently isolated from seed of capsicum annum and seed borne infection induced wilt in transplanted plants. Fruit rot was also common.

Dempseu and Chandler (1963) inoculated fresh harvested paper seeds with *Colletotrichum capsicum* and *Xanthomonas vesicatoria* and then gave various soak treatments. Agrimycin 500 was equally safe but not effective against fungi. The sesmesan, mercuric chloride and panogen 15 were effective but all reduced seedling vigour and higher doses of panogen 15 reduced total germination.

*Cladosperium*, *Cladosorides*, *Fusarium moniliformae*, *Aspergillus niger*, *Penicillium chrysogenum*, *Alternaria alternata*, *curvularia*, *phoma pumosum*, *Colletotrichum Sp.*, *Alternaria solani*, and *Fusarium moniliformae*. *Alternaria alternata* *Fusarium Sp*. were recorded as externally as well as
internally seed borne fungus. Siddiqui et al. (1977) reported primary source of collectotrichum capsici infection to be through infected seeds, and plant debris, which caused anthracnose of chilli.

Vidhya Sekaran and Thyagarajan (1981) stated that Fusarium oxysporum, reduced seed germination, growth rate and fruit yield.

Grover and Bansal 1970 reported that seeds of chilli obtained from diseased and healthy fruits from the market carried collectotrichum capsici as internally and externally leading to damping off of seedlings that died after emergence which produced abundanted aceruvuli and spores which become a potential source of primary infection.

Raut and Rath (1972) studied seed mycflora of chilli and observed different fungi viz Alternaria tenuis, Collectotrichum capsici, Sclerotium bataticola, Aspergillus niger, Penicillium, Rhizopus nigricans, Chourasia (1976) reported Alternaria SP, Collectotrichum capsici, phomaspp, Helmintho sporium Sp. and several other fungi associated chilli seeds at Jabalpur.

Pandey (1976) studied fungi associated chilli seeds by agar plats method. He isolated common fungi, viz. cladosporium clado sporades., F. moniliforme A. niger, penicillium, Altenaria alternata curvularia ohoma molteus, collectotrichum Sp, Alt. solani and fusarium A. Alternata, colletotrium Sp, A. solani and Fusarium Sp. was recorded as externally, seed borne.
DAUCUS CARROTA

It a biennial, that was known to the ancient Greeks and Romans but was not introduced into other parts of Europe until the middle ages. D.Carota var,Sativa,is native to Europe, Asia and North Africa, It is very doubtful whether the carrot is also native to the America, It appears that it was introduced from Europe early in the seventeenth century, in Verginia, and that it become popular only among the American Indians who spread it over the whole New continent.

The edible part of the carrot is its underground fleshy structure, consisting mainly of the swollen base of the tap root.

The carrot root contains,yellow,red pigments and B-Carotene, the precuroser of vit.A.These carotenes are responsible for the colour of the corrot and are present in the plastids.

The following are the major seed borne pathogens of corrot, (Paul Neergaard, (1969) Alternaria porri. Fusarium Sp.
Alternaria readicina,( black root rot), cercospora carotae. ( cercospora blight and bacterial blight) Bacterial blight (Xanthomonas carotae) reduces seed yields quantitativly and qualitatively.

Alternaria radicincause seed rot in carrot. Alternaria radicina, causes a black rot on root reducing their market value in quality as well as quantity. (Paul Neergaard 1978,Thomas 1943, Saponard 1964).

The seedborne mycoflora of cultivars obtained form Sindh Pakistan, were investigated using blotters, deep freezing and agar plate Methods, revealed pathogens which included Alternaria,
alternata, cladaosporium cladosporoides, Fusarium moniliforme.
A wenti, Chaetomium globosum.

Alternaria porri, F.Sp. develops carrot leaf blight and is widely seed transmitted. Alternaria radicina, cercospora carotae
Sclerotinia sclerotiorum Xanthomonas carotae, Erwinia Carotivora C.
Carotae-Cercospora have also been associated with seeds of carrot.

LYCOPERSICUM ESCULENTUM
Tomato is the fruit (berry) of Lycopersicum esculentum a member
family solanaceae.

The fruit is usually more or less spherical in shape but in
one variety it is pear shaped. Seed borne damage included, leaf
spots and defoliation, fruit rot, stem rot, wilt and mosaic, major
pathogens are, Alternaria porri, F.Sp. solani, (early blight)
Fusarium oxysporum F.Sp. lycopersici (fusarium wilt) Phytophthora
infestans (late blight) Verticillium dahliae, (verticillium
wilt) and Xanthomonas vesicatoria (Bacterial Canker) 15% loses
are due to seed borne diseases. Verticillium wilt is considerably
more wide spread than fusarium wilt. (Neergaard 1978)

In Florida less than 1% seed borne infection. Can cause an
epidemic Bacterial canker (Corynebacterium michiganense), has
caused serious losses in North America, Europe, Australia, and
Newzealand.

Dwiwedi and pathak (1978) reported that seed treatment with
difolatan even at its lower concentration (0.1) cersan and thiram
inhibited the toatl mycoflora at 0-2% and 0.3% seeds.
Walker (1976) reported that *Fusarium oxysporum*, *Fusarium lycopersici* were the causal organisms of wilt of Tomato, which is transmitted through seeds. Sharma and Soni (1975) stated the Phytophthora parasitica is externally and internally seed borne fungus of Tomato seeds which caused buckeye rot of Tomato.

Dwivedi and Pathak (1978) reported 25 fungi from the seeds of mogglove variety of Tomato amongst which most common were *Aspergillus*, *Rhizopus*, *Penicillium*, *Curvularia*, *Orlova* et al. (1982) observed 23 spp. of fungi out of which 15 were located from Tomato seed surface and 8 from the plant parts. *Penicillium* Sp. were predominant on the surface.

**Solanum melongena**

It is a perennial plant with a spreading or erect stem, up to 1.50 cm tall, and is probably native to India. Some authors believe that a description of European origin dating from the fifth century A.D. refers to the egg-plant but the ancient nations of the Mediterranean region hardly knew this vegetable.

The edible part is the fruit, which is berry, usually egg shaped with a smooth & shiny, surface deep purple in colour. It seems likely that egg-plant was introduced from India by Arabs into Spain and Africa, and today it is cultivated in almost all tropical and subtropical regions and also in warmer temperate regions, e.g. Southern Europe, and the southern states of the U.S.A. Egg-Plant is most important in India and the Far East.

Following may be considered as major seed borne pathogens.

*Alternaria tenuis,* (leaf spot and fruit attacking young seedlings in India.) *Colletotrichum melanocephalum* - (anthracnose)

*Colletotrichum melanocephalum*, *Rhizoctonia solani* (Damping off.)
Fusarium spp, Fusarium solani F. oxysporum, and F. moniliforme.
Sclerotinia sclerotiorum. Verticillium Albo-atrum wilt.
(Nobel & Richardson 1968) Sarode and Kadam, (1977) proved that seed treatment with thiram was the best control against Helminthosporium Sp. Constantly isolated from egg plant seeds.

Sen and Kapoor (1974) reported that Fusarium oxysporum, Fusarium lycopersici were controlled with seed dressing of brinjal with Bavistin which was followed Pathogen and good growth and higher yields.

*SORGHUM VULGARE*

Sorghum is an important human food crop in China, India and Africa being used to make porridge and bread. In other countries the plant and grain are mainly used for fodder. Some care is necessary while using it as fodder since the young green parts of the plant are edible and contain appreciable quantities of cyanide.

The grain of sorghum is sometimes used for brewing beer and the varieties with brown to purple colouring are mainly used for this purpose. There use is widespread in Africa especially in Tanzania, central Africa and south Africa. The alcohol content varies from 2% but often much stronger beers are brewed (up to 10% alcohol).

The following have been reported as major seed borne pathogens.

*Claviceps microcephala, Fusarium moniliforme, Bloseocercospora sorghi, (Zonate leaf spot) Periconia circinata, (Milo disease) Sphacelotheca cruenta (loose smut) S. Sorghi (kernel smut).*

Govindswamy et. al. (1975) in a study of 14 samples of *Sorghum* from regional millet stations, Coimbatore, Kovilpatti, Palur and Ariyalur examined. They reported presence of saprophytic moulds *Aspergillus niger, Rhizopus nigricans, Curvularia lunata* and well knows *Macro phomina, phaseoli, Fusarium moniliforme* and two other fungi *Helminthosporium* and *Pellicularia - feldamentosa*. Narasimnan Et.al. (1961) isolated 17 genera of fungi, *Aspergillus, Penicillium, Mucor, Rhizopus curvularia, Helminthosporium, Bofrytis, Cladascoprium, Phytopthora, Tricothelium, Chaetomium, Pucnidium, Apriocarpella* from *Sorghum* grain.

Juneja and Malik (1967) carried out survey in West Pakistan to detect the fungi associated with *Sorghum* seeds and
found *Aspergillus, Helminthosporium, Curvularia,* and *Fusarium,* to be seed borne. Siddiqui et al. (1974) studied the fungal flora associated with seeds of vegetables and cereals. They examined 28 samples of Sorghum for the presence of fungi and recorded % infection by *Alternaria tenuis* (20-38%), *Aspergillus* Sp. (12-16%), *Cladosporium cladosporodes* (1-3%), *Curvularia cymbopogonis* (0-5%), *Curvularia lunata* (1-6%), *Drechslera rostrata* (1-3%), *Tetramera cepricoccum parparascens* (0-2%), *Fusarium moniliforme* (0-1%), *Penicillium Spp.* (10-28%) and *Phoma* (1-2%). Pathogenicity was tested by the procedure followed by Shukla et al. (1980) on eleven fungi viz. *Asp. niger, A. flavus, A. ochraceous, Penicillium, Cyclopium, Curvularia lunata, Fusarium Sp. Alternaria, Chaetomium globosum, Rhizopus stolonifer* were isolated from the seed. The maximum number of fungi were recorded by blotter method.

The Fungal culture filtrates either inhibited or stimulated seed germination depending on the cultivar of sorghum. The culture filtrates adversely affected seedling vigour and root growth of the two varieties (Swarna and Neerujola) The growth and vigour of the two hybrids was not affected by most of the culture filtrates.

Total Amylase, $\alpha$- amylase and B-amylase content were dependent on. PH AT 5.0 PH a decrease in amylase activity in Sorghum which was observed (Wilkinson and Duncan 1990).

B-Amylase did not contribute to hydrolysis of starch granules.
Hydrolisis, however it contributed for starch granule hydrolysis primary via-its interactions with $\alpha$- amylase.
An acid endo peptidase has been purified from germinating sorghum seed by Garg and Virupaksha (1970). This enzyme was not sensitive to sulf hydryl group inhibitors.

Sorghum cultivars were artificially inoculated with Fusarium monili forme., (Giberella fujikuroi) conidia at flowering and bagged, these samples and untreated controls were harvested at physiological maturity and 2 weeks after (physiological maturity). The grains obtained from both treatments were studied for their milling, malting, and popping characteristics. Grains harvested at physiological maturity possessed superior milling and malting, characteristics where as late harvested, grains exhibited better popping characteristics milling and popping reduced the seed mycoflora considerably. (Bewley and Black 1978)

Carbohydrates that accumulate in developing seeds can be oligosaccharides or polysaccharides, the major oligosaccharides is sucrose, but a number of galactosyl sucrose and fructofuranosyl. Sucrose oligosaccharides are also found Polysaccharides. May combine more than one function. The most common storage polysaccharide is starch which serves only as a glucose reserve. A number of polysaccharides whose role appears to be structural are also depolymerised after imbibition and the released monosaccharides utilized by the seedlings.

Seeds characteristically contain relatively large amount of food reserves which support growth and development of seedling, until it can establish itself as a photosynthesizing autotrophic plant.
Inhibition of seeds of lipid, starch, protein reserves could be related with seedling vigour, and viability of seed lots. Protein synthesis starts within minutes after imbibition in some species after hours.

Glutamine is derived from the phloem stream and ketoglutarate is a product of carbohydrate, respiration. Glutamate synthase activity has been positively correlated with nitrogen accumulation in developing maize. The glutamate formed could subsequently be used in the synthesis of other amino acids by means of transaminase enzymes known to be present in immature cereal endosperm, and also most in developing seeds.

Any ammonium in reaching the seed may be metabolized by either glutamate, dehydrogenase or glutamine synthetase to give the amino acids glutamate/ Glutamine respectively. Certainly the transaminases which utilize glutamate for the synthesis of Aspartate and alanine are present in developing barley grains.

In turn Aspartate is itself a precursor of the essential amino acids threonine, methionine, lysine and isoleucine.

The aromatic amino acids tryptophan, tyrosine and phenylalanine also essential amino acids. Are probably derived from the carbon substrates Erythrose 4-phosphate and phosphoenolpyruvate which are intermediates of carbohydrate degradation. The nitrogen comes either from glutamine or from other amino acids by transamination. (Khan 1977).

It was concluded that I.A.A. was associated with the Germination processes rather than playing any significant role in overcoming dormancy.
Imbibition of water by Quiscent or dormant seed initiates biochemical activities which involve hydrolytic enzymes. Hydrolases may be pre existent or are synthesized of necessity. Protein synthesizing, machinery is activated and Oxidative metabolism dominates during germination.

As germination progresses the synthesis of new mRNA commences and additional protein are synthesized. The protein reserves in cereals are mainly found in the endosperm with a small amount around 2.5% in the embryo in contrast, there is usually little or endosperm in mature seed and the protein reserves are almost entirely confined, to the cotyledons, Haydecker Etal. (1975) primed seeds of carrot & onion with polyethylene glycol and found that emergence was early and Germination % high (Haydecker 1975)

Growth harmones accumulate in developing seeds in preparation of their subsequent role in controlling seed germination and seedling growth. Auxins and GA are bound in conjugated forms and are released during germination.

The surface of the seed is partly in contact with soil and partly in contact with soil pores. For emergence of radicle, cell-wall loosening is essential and this is accomplished by cell-wall degrading enzymes stimulated by auxins. Hormones increase in concentration before radicle emergence and this supports role of growth of regulators (Bewley and Black 1978).

A very early metabolic event during imbibition occurring in less than 15 min. appears to be reformation of keto acids from amino acids by deamination and transamination.
Germination in some seeds is enhanced when respiratory steps (terminal oxidation processes) are inhibited by DNP or KCN. DNP is an uncoupler of oxidation and phosphorylation and stimulation of germination is a Paradox. (Ross 1960)

Dry seeds contain many enzymes, most of these enzymes are resistant to disease and become fully active as soon as imbibition starts. A few enzymes, already present in the seeds undergo activation process at some stage during germination and become fully active as result of proteolysis.

Gibberellic Acid activates several enzymes during germination. Hydrolysis of storage lipids was accelerated by GA, sugar accumulation increased and hydrolysis of amygdaline was evident. (Kawecki, 1970, a,b,c).

GA also stimulated activity of phosphatase in apple embryos. Reports on GA indicated that it does not play any significant role in dormancy breathing, rather it is involved in metabolic events associated with germination and seedling growth.

Germination and the growth which it initiates involve a variety of biochemical reactions. It therefore not surprising that various chemicals affect final outcome of germination. Chemicals with regulatory activity assume importance here.

Nitrate ions have stimulatory effect on germination. Calcium promotes germination by binding with Uronic acid carboxyls.

Bioactive chemicals with or without growth regulating activity may improve seed performance. Seed protectants, fertilizers, pesticides and actidione belong to this group.

Infusion of bioactive compounds is usually done through
Compounds in solvents as surry as suspension, as spray, as dust, by incorporation in coats, pellets around seeds, via organic solvents.

Brewing industry early recognised the possible advantage of using GA for promoting enzyme activity in malting barley. The hormone promotes synthesis, release or both of amylase, proteinases and cellulases which break down starch, proteins and walls of cells of endosperm into mixture of sugars, polypeptides and amino acids. (Lang A, 1970).

Amylase is present in many plants. Typically in germinating cereals and in molds and bacteria. It in a Ca\(^{++}\) dependent enzyme.

Aspartic acid and Glutamic acids are dicarboxylic amino acids.

Assimilated nitrogen accumulates predominantly in glutamic acid (Chibnall, 1939). Recent studies have shown that ammonia, nitrate and elementary nitrogen enter via Glutamic acid, Glutamine, aspartic acid alanine. Glutamic acid is synthesized via reductive aminatuum of keto glutarate.

Considerable indirect evidence suggests that plant transaminases are also pyridoxal phosphate dependent enzymes. With wheat and cauliflower transaminases revealed a partial dependence upon pyridoxal phosphate (Ellis and Davis, 1961).

Endosperm break down in celery involves wall hydrolysis and cell separation. As in barely aleuron cellwall hydrolysing enzymes originate from the protoplasts of the same cell. GA stimulates wall hydrolysis.
Wall hydrolysis can be detected through release of wall-material in bathing medium. Arabinose and xylose containing compounds and free sugars appear within 10 hours in GA treated seeds. Xylanase, Xylopyranosidase and arabinofuranosidase are released. Endoxylanase releases monosaccharides.

An acid endo peptidase has been purified from germination sorghum seed by garg and Virupaksha (1970 a.b.). This enzyme was not sensitive to sulf hydryl group inhibitors.

Following imbibition of water a complex series of metabolic events initiates at the seed surface which becomes seed – Soil interface when seed is sown. Release of seed enzymes for degradation of seed reserve is influenced by seed borne fungi and bacteria which in turn are subject to impact of prevailing soil conditions. (Deshpande and Muley 1990) studies of seed enzymes seed exudates, and metabolites of seed borne pathogens (Kulkarni, 1979, Muley 1990) indicated that dominance of seed borne fungi depends upon nature of seed exudates. Germination of seeds and emergence of seedling is influenced by fungi. Inhibiting seed surface and individually or in combinations. This complex is again subject to influence by soil factors, biotic or abiotic. Efficiency of seed treatment chemicals depends largely on soil microbes. Experiments were under taken to asses the possibility of inducing desired changes in post imbibition phases to effect early germination and early emergence of seedling.
MATERIAL AND GENERAL METHODS

1) SOURCE OF CULTIVARS
Seeds of seven plants cultivated in this area were collected from local market for the study of seed health testing, and seed enzymes. Samples were separately collected in sterilized plastic containers and were stored without any treatment with fungicides or insecticides. Viability tests were conducted periodically. Standard blotter tests were used in seed health testing, using every time 400 seeds. Observations were recorded for percentage infection by individual seed born pathogen, its association with non-germinated seeds and distribution on seed surface. Fungi and bacteria which appeared on seed were isolated in pure culture for identification and for further study.

The following cultivars were investigated for seed microflora and study of enzyme release pattern.
1) Allium cepa Linn. (Onion) Nasik red variety.
2) Brassica oleracea Linn. (Cauliflower ) Hind selected 'katki'.
3) Capsicum annuum Linn. (Chilly) "Safal" improved chilly seeds.
4) Daucus carota Linn. (carrot) Local Seeds.
5) Lycopersicum esculentum Linn. (Tomato) "Safal" improved seeds of Tomato.
6) Solanum melanogena Linn. (Brinjal) "Vaishali"
7) Sorghum vulgare Linn. (Jowar) C-SH-I

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2) ASSESSMENT OF SEED MICROFLORA

Standard blotter test: - 400 seeds were equidistantly spaced out on moist sterile blotters with and without different pretreatments, as required in petriplate moist chambers. 20 petriplates of 9 cm. diameter each containing 20 seeds were nincubated at 28 +/- 2°C for 96 hours. Observations were made for fungi and bacteria appearing on seeds every 24 hours, and growth, was carefully transferred to potato dextrose agar (PDA), Malt Extract Agar (MA) or Nutrient agar (N.A.), as required.

NaOCl Treatments: - 400 seeds of each variety were kept immersed in 1% NaOCl or test chemicals solution in beaker or conical flasks for 10 minutes, the flasks were shaken continuously on a rotary shaker. Soaked seeds were spaced out on moist sterile blotters or cotton pads in petriplate moist chambers, after thorough washing with sterile Distilled water. 20 petriplates of 9 cm. diameters each containing 20 seeds were incubated at 28 +/- 2°C, upto 96 hours. After seedling development the wet pads with seeds were squeezed gently well, and solution was filtered through whatman filter paper. A clear extract was obtained. It was used as such or after appropriate dilution as source of enzymes.

3) MEDIA.

The following media were used in various experiments on growth and enzyme production. For solidifying media 20 gm. of Agar was added to one litre of the medium wherever necessary.
1) Potato Dextrose Agar. (PDA)

Peeled Potato - 250 gm.
Glucose - 20 gm.
Agar - 15 gm.
Distilled water 0- 1000 ml.

2) Nutrient Agar. (NA)

Peptone - 5.0 gm/lit.
Yeast extract - 1.5 gm./lit.
Beef extract - 1.5 gm/lit., Agar 20 gm.
Sodium Chloride - 15.0

3) Malt extract (MA)

Peeled potatoes - 60 gm.
Malt extract - 20 gm.
Difco. bact. Agar - 20 gm.
Distilled water - 250 m.

4) GLUCOSE NITRATE MEDIUM.

Glucose 10.00 gm.
KNO₃  02.50 gm.
KH₂PO₄  01.00 gm.
MgSO₄·7H₂O  0.50 gm.
D.W. 1000 ml.
Agar 20 gm.

5) Calcium phosphate Agar -(2%)
Calcium phosphate -2 gm.
Agar - 20 gm.

Distilled water - 250 ml.

Sterilization - All the media used in this study were sterilized at 15 lbs pressure for 20 minutes, in an Autoclave before use. Liquid Media were placed in conical flasks, (250 ml.) and were sterilized.

GLASS WARES - All the glasswares were thoroughly cleaned with acid dichromate cleaning mixture first, then hot boiled tap water, rinsed with distilled water and dried in oven before use.

ISOLATION, IDENTIFICATION AND PRESERVATION OF FUNGAL CULTURES.

For identification of the fungi associated with seeds Semipermanent slides were prepared at appropriate stage of growth of the fungus and measurments of hyphae, conidia etc. were taken. These were then compared with data recorded earlier in various sources and identification was confirmed. Neergaard 1980, Barnett 1951, Ellis 1979, Subramanyam 1963. Cultures of fungi were preserved on PDA, GNA and malt extract slants for further study. Subculturing was done periodically to ensure viability. Bacterial cultures were maintained on N.A. slants.

Fresh isolations were made directly from the stored seed, periodically. Seed herbaria were maintained by preserving the seeds with the fungus on filter paper with the help of transparent adhesive tape.

All the cultures were incubated at 15°C, and subcultures were made after every 15 days.

Inoculum - Spore suspension from fungal cultures, was
prepared by adding sufficient sterile water to eight day old PDA slant culture. 2.5 ml. of Spore suspension was added to 25 ml. medium in flasks. For bacteria nutrient broth cultures in 10 ml. lots were used after appropriate dilution.

INCUBATION - Both fungal and bacterial cultures were incubated at 28 +/-2°C in the laboratory and variation in temperatures was recorded during the entire experimental work under study. In all experiments fungal cultures were incubated for 8 days. and bacterial cultures were incubated for 48h. Results have been presented after repeating experiments.

PREPARATION OF SPORE SUSPENSION.

In some experiments spore cell suspensions were used. The fungus was grown as lawn cultures in petriplates (9cm. dia.) for 8 days, sterile water was added to each plate and spore suspensions from two plates was made upto 150ml. with sterile distilled water.

In the same way cell suspension was prepared from bacteria grown on N.A. plates for 48h. Cell suspension was prepared by adding sterile distilled water to each plate.

CHRROMATOGRAPHY

Chromatography were used for detecting amino acids and sugars released by action of seed enzymes. 0.05 ml. samples were applied 4cm. apart on whatman paper. Spots were given using capillary tubes. To detect amino acids and carbohydrates following solvent system was used.
N-butyl alcohol - 40 ml.
Glacial acetic acid - 10 ml.
Distilled water - 50 ml.

The solvent mixture was separated using separating funnel and lower layer was placed at the bottom of the chromatographic cabinet. The spotted paper was then allowed to equilibrate for 4 hours and then the upper fraction was poured into the trough of chromatographic cabinet. This was allowed to irrigate the paper for 8 hours. It was then dried and sprayed with reactant as detector.

For amino acids.
---------------------
400 mg. ninhydrin in 100 ml. acetone.

For carbohydrates (Sugars.)
--------------------------
Benzidine spray mixture prepared as
Benzidine - 0.5gm.
Glacial acetic acid - 100 ml.
45% Trichloro acetic acid 10.0 ml.
95% alcohol 100.0 ml.

For amino acid detection papers were heated for five minutes by hair drier and for reducing sugars- papers were kept in hot chamber at 70°C. Identification was done by visual comparison with spots developed by known amino acids/sugars on chromatograms processed similarly.
TESTS FOR DETECTION OF ENZYMES.

Of the following enzymes Tests were conducted for production and activity of the following enzymes.

1. **Aminotransferase** (2.6.1) transaminase
2. Aspartic acid, aminotransferase,
3. **Amylase** (3.2.1)
4. **B-Fructofuranosidase** (invertase, sucrase, saccharase) (3.2.1.2.6. B-D- Fructo Furanoside Fructohydrolase)
5. **B-Glucosidase** (Cellobiase) (Gentiobiase, emulsin 3.2.1.21 B-D- Glucoside glucohydrolase)
6. **Catalase** (1.11.1.6-Hydrogenperoxide, Hydrogenperoxide oxydoreductase)
7. Cellulase Glucanase (3.2.1.4 B -Glucan4 - glucanohydrolase),
8. Pectinase
9. Catalase.

ESTIMATION OF GLUCOSE.

Five ml. of Fehling's solution A were mixed with 5ml. of Fehlings solution B, in a beaker and the mixture was made upto 50ml. by adding 40ml. of distilled water to it. The culture filtrate in the burette was run down 1ml. at a time and the mixture was boiled and stirred, continuously. The culture filtrate was added until the blue colour of Fehling's solution, disappeared or a red precipitate formed. A drop of K₄FeC₅N₆ was then added to this decolourised solution to confirm whether complete reduction took
place or not i.e. non formation of red precipitate again. The amount of glucose in the C.F. was calculated as follows.

10 ml. of fehling's sol\(^n\) = 0.05 gm of glucose.

Let the burette reading or amount of culture filtrate be = X ml. In the given quantity of glucose solution (C.F.), the amount of glucose will be = given quantity \(\times 0.05\)

\[\frac{X}{ml.}\]

Benedict's quantitative and qualitative solutions were also used, to confirm results obtained by Fehlings test.

**Test for Amylase** (3.2.1)

To five ml. of 0.1% starch sol\(^n\), 5 ml of seed leachate (enzyme sol\(^n\)) was added, the mixture was thoroughly shaken and the tubes were incubated at 28 +/- 2\(^\circ\)C. for 24 h.

After 24 h, the amount of glucose/reducing sugars was made out using fehling's sol\(^n\).

**Cellulase-Glucanase** (3.2.1.4 B - Glucan 4 - Glucano Hydrolase)

To five ml. of 0.1% C.M.C. sol\(^n\), 5 ml of seed leachate (enzyme sol\(^n\)) was added, the mixture was thoroughly shaken, and the tubes were incubated at 28 ( +/- ) 2\(^\circ\)C. for 24 hrs.

After 24 hrs, the amount of glucose/reducing sugars was made out using fehling's sol\(^n\) (A+B).

**Pectinase** (Polygalacturonase, Pectinase (3.2.1.15)

Polygalacturonide glucanohydrolase)

To five ml. of 0.1% pectine sol\(^n\), 5 ml of seed leachates (enzyme sol\(^n\)) was added, the mixture was thoroughly shaken and the tubes were incubated at 28 +/- 2\(^\circ\)C. for 24 hrs.
After 24 hrs. the amount of galacturonic acid reducing groups was made out using fehling's sol^N_1 (A+B). The results were confirmed with benzidine reagent.

Protease - (proteinases, peptidases, gelatinase)

To five ml. of 0.1% gelatine sol^N_1 5 ml. of seed leachates (enzyme sol^N_1) was added. The mixture was thoroughly shaken and the tubes were incubated at 28 +/- 2°C for 24 hrs. the amount of protease action products i.e. amino acids release were tested by adding 0.4% Ninhydrin solution. The solutions were heated wherever necessary and again incubated for 24th or for 48 and 72 hrs.

Test For B-Glucosidase - (cellobiase, gentiobiase, emulsin (3.2.1.21 B-D Glucoside Glucohydrolase)

To five ml. of 0.1% cellobiose or Salicin sol^N_1 5 ml. of seed extracts (enzyme sol^N_1) was added. The mixture was thoroughly shaken and the tubes were incubated at 28 +/- 2°C for 24 hrs. After 24 hrs. the amount of glucose reducing sugars was made out using fehling's test.

**TEST FOR B-FRUCTO FURanosIDASE** (Sucrase, invertase, saccharase) (3.2.1.26 B-D Fructo furanoside fructohydrolase)

To 5 ml. of 1% Sucrose solution. 5 ml. of seed leachates (enzyme solution) was added. The mixture was thoroughly shaken and the tubes were incubated at 28(+/−)2°C for 24 hrs. After 24 hrs. the amount of glucose/reducing sugars was made out using Fehling's test.

**TEST FOR ASPARTIC ACID ‘AT’** (Amino transferase / transaminase)

To 5 ml. 0.1% Aspartic acid solution, 5 ml. of seed enzyme solution was added. The mixture was thoroughly shaken and the tubes were
incubated at 28 +/− 2°C for 24 hrs. After 24 hours, the amount of Aspartic ‘AT’ was tested by adding 0.4% Ninhydrin solution and intensity of color was noted the solutions were heated wherever necessary, and again incubated for 24 hrs. Transaminase action products were detected by Chromatography also.

TEST FOR GLUTAMIC ACID ‘AT’. (Amino transferase, transaminase)

To 5ml. of 0.1% Glutamic Acid Solution. 5ml. of seed extracts (Enzyme Solution) was added, the mixture was thoroughly shaken and the tubes were incubated at 28+/− 2°C. For 24hrs. After 24hrs. the amount of glutamic ‘AT’ released were tested by adding 0.4% ninhydrin solution. The solutions are heated wherever necessary and again incubated for 24hrs. at 48 and 72 hrs. And tests were conducted. Formation of other amino acids/release of NH₄/CooH group was tested wherever possible.

TEST FOR CATALASE – (1:11:1.6-Hydrogen Peroxide:Hydrogen Peroxide oxidoreductase)

Moist seeds (Soaked in Water for 24hrs.) Seeds of all the test cultivars were cut into slices / sections as (0.1mm. in thickness) and were used.

Sections were placed on separate glass slide. A few drops of H₂O₂ (Hydrogen Peroxide Solution) were added, evolution of oxygen bubbles indicated the presence of catalase enzyme.
CHAPTER ONE

SEED HEALTH TESTING AND SEED ENZYMES

OF ALLIUM CEPA LINN.
Plate I

Seed Health testing and seed enzymes of *Allium cepa Linn*

cv Nashik red
Seed Health Testing and Seed Enzymes of Allium cepa

Onion seeds (Allium cepa Linn. Cultivar Nasik Red.) were tested for seed borne fungi and bacteria and their effect on seedling growth. Seeds were incubated on cotton pads at 28±/-2°C following treatment with test chemicals for a period of four days. Observations for percentage germination, seedling development, extent of damage measured in terms of percentage infection and for seed borne pathogens were recorded. Observations were done under Stereomicroscope and results are based on 400 seed lots in each experiment.

I) Effect of NaOCl

Seeds of Onion showed 20.0% germination in the control, and 27.5% when treated with NaOCl. Germination improved by 7.5% root length increased by 5mm. in treated seeds. While shoot length increased by 6mm. in treated seeds. 36.5% seeds were infected in the control where as only 5% showed infection following treatments.

Fusarium oxysporum and Pseudomonas cepacia were observed on seeds before and after treatment (Table 1.1)

Table 1.1 Seed Health Testing of Allium cepa Effect of NaOCl.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>20.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Root length (Cms)</td>
<td>3.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Shoot length (Cms)</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Infection (%)</td>
<td>36.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Fungi/Bacteria</td>
<td>F. oxysporum, Pseudomonas, cepacia.</td>
<td>F. oxysporum, Pseudomonas, cepacia.</td>
</tr>
</tbody>
</table>
Plate II Allium cepa

A. Allium cepa seeds infected by pseudomonas cepacia
Plate II A
ii) **Effect Of Stimulators Of Germination**

Treatment with Stimulators of germination improved germination and it was 100% in N.A.A & 2.4-D treated lots 95% in I.A.A. and G.A. treated lots, 85% in KNO₃ treated lots, and 40% thiourea treated seeds. Root length was decreased in all treatments except IAA. Maximum reduction was observed in 2.4-D. IAA, G.A. and KNO₃ stimulated shoot growth infection was reduced considerably in all cases however in thiourea 30% seeds were infected. And R. nigricans developed colonies on seeds. (Table 1.2)

<table>
<thead>
<tr>
<th><strong>Observations</strong></th>
<th>C</th>
<th>I.A.A.</th>
<th>G.A.</th>
<th>NAA.</th>
<th>2,4-D.</th>
<th>KNO₃</th>
<th>Thiourea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>20.0</td>
<td>95.0</td>
<td>95.0</td>
<td>100</td>
<td>100</td>
<td>85.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Root length (Cms)</td>
<td>3.3</td>
<td>3.3</td>
<td>2.0</td>
<td>2.0</td>
<td>0.5</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Shoot length (Cms)</td>
<td>1.3</td>
<td>3.1</td>
<td>2.0</td>
<td>1.0</td>
<td>1.1</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Infection (%)</td>
<td>36.5</td>
<td>5.0</td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Fungi/bacteria</td>
<td>F. oxysporum cepacia cepacia - cepacia nigricans P. cepacia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

iii) **Effect of inhibitors**

Germination improved both in DNP & KCN treated seeds of onion (100% in DNP & 60% in KCN). Root length and shoot length decreased considerably in 2.4 DNP treated seeds KCN improved shoot length. Fungi observed in seeds in the control, were absent following treatment. Infection was reduced to 0% in DNP and to 5% in KCN treated seeds. Only *Pseudomonas* was observed on treated seeds. (Table 1.3).
(Table 1.3).

**Seed Health Testing of Allium cepa - Effect of Inhibitors.**

<table>
<thead>
<tr>
<th>Observations</th>
<th>Control (Water)</th>
<th>DNP</th>
<th>KCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>20.0</td>
<td>100.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Root length (Cms.)</td>
<td>3.3</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Shoot length (Cms.)</td>
<td>1.3</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Infection (%)</td>
<td>36.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Fungi / Bacteria</td>
<td>Fusarium oxysporum.</td>
<td></td>
<td>Pseudomonas cepacia</td>
</tr>
<tr>
<td>Associated</td>
<td>Pseudomonas cepacia.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

iv) **Seed Enzymes. Effect of Infection**

Onion seeds secreted all the test enzymes and activity was expressed early (i.e. with 24 hours) in the Control. Glu-AT, Sucrase, Pectinase and Catalase activity was high. Asp-AT, B-glucosidase, Cellulase and protease activities were moderate and amylase activity was low.

Following Infection by Pseudomonas Activities of all Enzymes appeared late except for Catalase. B-Glucosidase Cellulase and protease activities improved whereas pectinase and Glu-AT decreased. (Table 1.4)
Table 1.4 Enzyme Release by Seeds of Allium cepa.

Effect of infection by Pseudomonas cepacia:

<table>
<thead>
<tr>
<th>Enzymes Tested</th>
<th>Control</th>
<th>Germination 50%</th>
<th>Infected Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotriose ferasue</td>
<td>E.M.</td>
<td>D.M.</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>E.H.</td>
<td>D.M.</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>E.L.</td>
<td>D.M.</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>E.H.</td>
<td>D.H.</td>
<td></td>
</tr>
<tr>
<td>B-Fructofuranosidase</td>
<td>E.M.</td>
<td>D.H.</td>
<td></td>
</tr>
<tr>
<td>B-Glucosidase</td>
<td>E.M.</td>
<td>D.H.</td>
<td></td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M.</td>
<td>D.H.</td>
<td></td>
</tr>
<tr>
<td>Pectinase</td>
<td>E.H.</td>
<td>D.M.</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>E.M.</td>
<td>D.H.</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>E.H.</td>
<td>E.M.</td>
<td></td>
</tr>
</tbody>
</table>

E=Early Production  D = Delayed Production,  L = Low activity
M = Moderate activity H = High activity

v) Effect of Stimulators.

Catalase activity remained unaltered following treatment by stimulators. Protease activity was delayed in IAA and was unchanged in other treatments. Pectinase appeared late in KNO₃ treated lots and activity was low in all except GA. Cellulase activity was low to moderate. All the treatments improved B-glucosidase activity. GA and KNO₃ reduced sucrase activity. Amylase activity also remained unchanged. (Table 1.5)
**Table 1.5 Enzyme Release by Seeds of Allium cepa.**

**Effect of Stimulators of Germination.**

<table>
<thead>
<tr>
<th>Enzymes tested</th>
<th>C. I.A.A. G.A. NAA Thiourea 2,4-D KNO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotransferase</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>E.L. E.M. E.M. E.M. E.L. E.M. E.M.</td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M. E.M. E.L. E.L. E.L. E.L. D.M.</td>
</tr>
<tr>
<td>Protease</td>
<td>E.M. D.M. E.M. E.M. D.M. E.M. D.M.</td>
</tr>
</tbody>
</table>

E = Early production, D = Delayed production,
L = Low activity, M = Moderate activity, H = High activity.
vi) Effect Of Vitamins

All the eight test enzymes were secreted by seeds of Allium cepa in the control and activity was expressed early. Treatment with vitamins inhibited Protease. Enzyme activity was early for all enzymes, following treatment, with Thiamine. High activity of Cellulase, B-Fructofuranosidase, Aminotransferase were observed. With Ascorbic acid treatment activities of all Enzymes were early and high. Aspartic acid AT activity was absent in Riboflavin and pyridoxin treated seeds. The activity was moderate except Pectinase where it was high in Riboflavin treated lots.

The Glutamic AT activity was absent and activity was high in cellulase and pectinase moderate in amylase & B-Fructofuranosidase and low in B-Glucosidase in Pyridoxine chloride treated lots. (Table 1.7).

(Table 1.7)

<table>
<thead>
<tr>
<th>Enzymes Tested</th>
<th>C. Thiamine</th>
<th>Ascorbic acid</th>
<th>Riboflavin</th>
<th>Pyridoxine Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotransferase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid AT</td>
<td>E.M. E.H.</td>
<td>E.H.</td>
<td>(Absent)</td>
<td>(Absent)</td>
</tr>
<tr>
<td>Glutamic acid AT</td>
<td>E.H. E.H.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>(Absent)</td>
</tr>
<tr>
<td>Amylase</td>
<td>E.L. E.M.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>B-Fructofuranosidase</td>
<td>E.H. E.H.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>B-Glucosidase</td>
<td>E.M. E.L.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.L.</td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M. E.H.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Pectinase</td>
<td>E.H. E.L.</td>
<td>E.H.</td>
<td>E.H. Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Protease</td>
<td>E.M. Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

E=Early Production D = Delayed Production, L = Low activity M = Moderate activity H = High activity
viii) Effect Of Salts

All the eight test enzymes were secreted by seeds of Allium cepa in the control and the activity was expressed early. Enzymes activity was expressed early in all the treatments except for protease which was absent in seeds treated with MgSO₄, calcium phosphate NH₄NO₃. Protease activity was low. Pectinase activity was high with (NH₄)₂SO₄, MgSO₄ and, Calcium Phosphate. Asp.-AT improved in all treatments. Glu.-AT was reduced in (NH₄)₂SO₄ and Tyrosine. Amylase activity remained unchanged following Tyrosine treatment and improved slightly in other salts. Low Sucrase activity was observed in all except MgSO₄ treatment. MgSO₄ and calcium phosphate improved B-Glucosidase activity. Ammonium sulphate improved cellulase. Pectinase activity was moderate in NH₄-NO₃ treated lots. (Table 1.8)

(Table 1.8)
Enzyme Release by Seeds of Allium cepa - Effect of Salts

<table>
<thead>
<tr>
<th>Enzymes Tested</th>
<th>C.</th>
<th>NH₄NO₃</th>
<th>(NH₄)₂SO₄</th>
<th>MgSO₄</th>
<th>Ca₂SO₄</th>
<th>(PO₄)₃</th>
<th>Tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotransferase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic acid AT</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.L.</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.M.</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>E.L.</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.L.</td>
<td></td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M.</td>
<td>E.L.</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.L.</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>E.M.</td>
<td>Absent</td>
<td>E.L.</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>E.M.</td>
</tr>
</tbody>
</table>

E=Early production, D=Delayed production, L=Low activity, M=Moderate activity, H=Activity High
ix) **Effect Of Organic Acids**

All the eight test enzymes were secreted by seeds of Allium cepa in the control and activity was expressed early. With Fumaric acid all test enzymes activity was early except for protease where it was absent high Cellulase, Pectinase and Aminotransferase activity was evident. With Citric acid treated lots Amylase activity was low and Aminotransferase, Cellulase pectinase activities high. B-Fructofuranosidase, B-Glucosidase and protease, activities were moderate. (Table 1.9)

(Table 1.9)

<table>
<thead>
<tr>
<th>Enzymes Tested</th>
<th>Control</th>
<th>citric acid</th>
<th>Fumaric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid AT</td>
<td>E.M.</td>
<td>E.H.</td>
<td>E.M.</td>
</tr>
<tr>
<td>Glutamic acid AT</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Amylase</td>
<td>E.L.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>B-Fructofuranosidase</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>B-Glucosidase</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M.</td>
<td>E.H.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Pectinase</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Protease.</td>
<td>E.M.</td>
<td>E.M.</td>
<td>Absent</td>
</tr>
</tbody>
</table>

E=Early production, D = Delayed Production, L = Low activity, M=Moderate activity, H= High activity.

x) **Effect Of Catabolites**

An experiment was set to see whether catabolite repression is operative at the level of seeds, Glucose, a catabolite of starch and
cellulose degradation, Galacturonic acid, a catabolite of pectin and two amino acids i.e. Aspartic and Glutamic acid, Catabolites of protein degradation were used in treatments. Corresponding Enzyme activities were assessed. The results are presented in Table 1.10. With Glucose, amylase and Cellulase activities were delayed and were moderate. With Aspartic and Glutamic acid, corresponding Enzymes appeared late and activities were moderate. Similarly Galacturonic acid delayed pectinase activity and it was moderate. (Table 1.10)

**Enzyme Release by Seeds of Allium cepa - Effect of Catabolites.**

<table>
<thead>
<tr>
<th>Catabolites used</th>
<th>Aspartic acid-AT</th>
<th>Glutamic acid-AT</th>
<th>Amylase acid-AT</th>
<th>Cellulase acid-AT</th>
<th>Pectinase acid-AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid AT</td>
<td>D.M.</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Glutamic acid AT</td>
<td>---</td>
<td>D.H.</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Glucose</td>
<td>---</td>
<td>---</td>
<td>D.M.</td>
<td>D.M.</td>
<td>---</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>D.M.</td>
</tr>
</tbody>
</table>

E=Early Production, D=Delayed Production, L=low activity, M=Moderate activity H=High activity

**xi) Effect Of inducers**

Simple sugar monomers act as repressors and at times inducers of polysaccharide degrading Enzymes. Glucose, a hexose and xylose a pentose were used to treat seeds of onion and enzyme activities were recorded. Early activity was observed in all cases with Glucose. High Asp.-AT, B-Glucosidase, and Pectinase, activities were recorded. With Xylose, Glutamic AT.B-Fructofuranosidase, and cellulase activities improved over the control. (Table 1.11)
Enzyme Release by Seeds of Allium cepa - Effect of inducers

<table>
<thead>
<tr>
<th>Enzymes Tested</th>
<th>Control</th>
<th>Glucose</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid AT</td>
<td>E.M.</td>
<td>E.H.</td>
<td>E.M.</td>
</tr>
<tr>
<td>Glutamic acid AT</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Amylase</td>
<td>E.L.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
<tr>
<td>B-Fructofuranosidase</td>
<td>E.H.</td>
<td>E.M.</td>
<td>E.H.</td>
</tr>
<tr>
<td>B-Glucosidase</td>
<td>E.M.</td>
<td>E.H.</td>
<td>E.M.</td>
</tr>
<tr>
<td>Cellulase</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.H.</td>
</tr>
<tr>
<td>Pectinase</td>
<td>E.H.</td>
<td>E.H.</td>
<td>E.M.</td>
</tr>
<tr>
<td>Protease</td>
<td>E.M.</td>
<td>E.M.</td>
<td>E.M.</td>
</tr>
</tbody>
</table>

E = Early Production,  D = Delayed Production,  L=Low activity,
M = Moderate activity,  H = High Activity