The findings in this thesis are based on investigations carried out by the candidate at the Department of Botany, Faculty of Science, Dayalbagh Educational Institute (Deemed University) Dayalbagh, Agra during the year 1994-97.

The present work was taken up with the aim and objective of obtaining the true potato seeds by raising grafted potato plants on tomato stocks for profuse flowering that lead to fruit and seed formation emphasizing the following: (i) The study of condition of stigma and style for pollen tube growth, pollen number, pollen germinability and viability. (ii) biochemical, histochemical and enzymological studies of stigma, pollen, transmitting tissue, anther and ovule; (iii) developing methods for production of best embryo-types within true seeds and their germination for better seedlings.

For such a work flowers were the pre-requisite and flowering genotypes selected for obtaining true potato seeds were MST, Kufri Jyoti and Kufri Badshah of Solanum tuberosum ssp. tuberosum.

Several techniques and parameters were used that included field and laboratory studies; such as brick planting, grafting, repeated hand pollinations, quantitation, dissections and microtechnique. The representation of observations and results had been done by graphs, histograms, free-hand sketches, camera lucida drawings and photographs.

Usual techniques and reagents for qualitative and quantitative microchemical tests had been used for reducing sugars, starch, pectic compounds, lipids and proteins. For amino acid determination, paper chromatography technique was performed and Rf values calculated.

Histochemical studies were performed using hand dissections and microtome paraffin-wax sections of reproductive parts of potato plant. The tests for proteins, polysaccharides, lipids, nucleic acids and certain enzymes were performed using various stains (dyes) for particular reserves.

Electrophoretic technique had been employed for presence and separation of total proteins, glycoproteins lipoproteins, nucleic acids and
The extract of ripe fruit showed maximum concentration of reducing sugars revealing the optical density and transmittance.
certain enzymes by their specific stains in vegetative and reproductive parts of potato plant.

The extract of ripe fruit showed maximum concentration by revealing 0.37 and 43.0 optical density(O.D) and % transmittance. This had been closely followed by sprouted tuber and aerial tuber where O.D and % transmittance revealed 0.36 and 44.0; 0.35 and 45 respectively. The extract of pollen grains and unripe fruits showed slightly less amount with 0.34 and 46.0; 0.34 and 46.0 as O.D and of % transmittance. The extract of stigma underground tuber and anther revealed further decrease in concentration with 0.335 and 46.5; O.D and % transmittance. The least concentration was found in calyx with 0.315 and 48.5 as O.D. and % transmittance respectively.

The test for pectic compounds indicated its high concentration in the wall of the pollen tube cap closely followed by pollen tube wall behind it, ripe fruit and transmitting tissue of style.

The tests for starch present in the extract of underground tuber anther, ripe and unripe fruit showed highest concentration and larger sized starch grains. The style and stigma at stages 9 and 12 of floral bud revealed least concentration with medium sized starch grains.

High amount of total proteins were found in extract of aerial tuber as 4.815 μg/ml whereas, slightly low amount in underground tuber as 3.750 μg/ml. The least amount of total proteins, however was revealed as 0.566 μg/ml by stylar extract.

The underground tuber contained highest number of amino acids being 12. The aerial tuber and sprouted tuber revealed presence of 10 and 9 amino acids respectively. Interestingly, there was a uniformity in number of amino acids contained in pollen, ovary and anther (8 amino acids) with one more (9 amino acids) in style at stage 12 of the floral bud.

The anther contained 6 amino acids and ovary showed 4 amino acids (least in number); stigma contained 9 whereas 8 amino acids each in style and pollen.

The amino acid proline showed its occurrence in all the
vegetative and reproductive parts studied except in the stylar tissue of stage 12 of floral bud whereas an amino acid glutamine occurred only in underground sprouted tuber. The aromatic amino acid tryptophan occurred in all parts of stages 9 and 12 except in ovary of stage 9 and unripe and ripe fruit and seeds.

Seven amino acids were commonly present in pollen tetrad and pollen as asparagine, aspartic acids, histidine, leucine, proline, tryptophan and valine. The eight amino acid contained by pollen was tyrosine.

The stigma at the two stages 9 and 12 of floral bud development showed presence of same free amino acids asparagine, aspartic acid, histidine, isoleucine, leucine, proline, tryptophan, tyrosine and valine. Style of stage 9 contained asparagine, glycine, histidine, leucine, proline, threonine whereas, at stage 12 of floral bud the stigmatic papillae showed presence of glycine, histidine, leucine, lysine, threonine, tryptophan and tyrosine.

The free amino acids at stage 12 of floral bud present in ovary and anther were asparagine, aspartic acids, isoleucine, leucine, phenylalanine, proline tryptophan and valine and as paragine, aspartic acid, isoleucine, phenylalaine, proline, serine, tryptophan and valine respectively.

Histochemical tests performed for protein showed significantly high concentration in the cells of epidermis and middle layer cells of the wall of pollen tetrad and their nuclei and tapetal cells at stage 9 of floral bud. The wall and content of the pollen grains, stigmatic papillae, transmitting tissue cells at stage 12 of floral bud revealed prominent activity and localization of total proteins. Endosperm and cotyledons of embryo showed similar localization. The epidermal cells of anther, middle layer cells of stage 12 of floral bud, the nuclei of embryo-sac, cells of transmitting tissue of stage 9 of floral bud, however, revealed slightly low concentration. The cytoplasm of anther epidermal cells middle layer cells, stigmatic cells, nucellus and integument at stage 9 of the floral bud revealed decreased concentration of proteins.

Significant localization for polysaccharides was revealed in the stage 12 of the floral bud the epidermal cells, middle layer cells of anther and their nuclei, exine of pollen grains and their nuclei, outer layer of
stimatic papillae and their nuclei; transmitting tissue cell and nuclei of embryo-sac revealed. In the stage 9 of floral bud epidermal cells of anther, wall of pollen tetrad and their nuclei, stigmatic cells, transmitting tissue cells and nuclei of embryo-sac showed moderate distribution of polysaccharides. In the stages 9 and 12 of floral bud cytoplasm of epidermal cells, middle layer cells, pollen tetrad, cortical tissue of style, nucellus and integument showed very low activity. The embryo and seed coat cells, however revealed decreased concentration.

Test for lipid revealed its presence in epidermal cells of anther, exine of pollen grain, wall of pollen tetrads and their nuclei at stages 9 and 12 of floral buds by localization of high concentration. The outer wall of stigmatic cells and their nuclei, nuclei of embryo-sac at stage 12 of floral bud, endosperm and cotyledons revealed similar distribution of lipids. The cytoplasm and nuclei of epidermal cells of anther wall and wall of the middle layer cells of stages 9 and 12 of the floral bud and the nuclei of middle layer cells at stage 9 showed slightly low concentration. The cytoplasm of pollen tetrad, stigmatic cells, cells of transmitting tissue, embryo-sac at stage 9 of floral bud, cells of integument at stages 9 and 12 and the seed-coat revealed still lower amount. The cytoplasm of middle layer cells at stage 9, the cytoplasm of stigmatic cells at stage 9 and 12 showed considerable low quantity of lipids.

Histochemical tests for nuclei acids revealed that in the stage 9 of the floral bud the nuclei of the epidermal cells of anther and nuclei of and nuclei of middle layer cells at stage 9 and 12 of anther, nuclei and cytoplasm of pollen tetrad, pollen grains, nuclei of stigmatic cells and nuclei of embryo-sac at stages 9 and 12 of floral bud revealed high concentration of RNA. The nuclei of epidermal cells of pollen tetrad stigmatic cells, and nuclei of embryo-sac at stage 9 revealed rich activity of DNA. The nuclei of embryo-sac at stage 12 of floral bud showed similar activity of DNA. The nuclei of middle layer cells, nuclei of pollen grains, nuclei of papillar cells of stigma at stage 12 of the floral bud showed moderate localization of DNA. The nuclei of middle layer, and nuclei of embryosac at stage 9 of floral bud revealed little distribution of DNA. The nuclei of
developing seed coat showed very little concentrations of RNA and DNA.

Test for peroxidase enzyme revealed that in the stage 9 of the floral bud the wall of epidermal cells of anther and nuclei of stages 9 and 12 of anther, outer wall and nuclei of pollen tetrad and pollen grains showed significantly high activity. The stigmatic cells with their nuclei at stages 9 and 12 of floral bud, content of transmitting tissue cells of stage 9, the nuclei of embryosac at stage 12 revealed prominently high localization of the enzyme. The wall of epidermal cells, cytoplasm of pollen tetrad, transmitting tissue cells at stage 12, the integuments, nucellus and embryo-sac at stages 9 and 12 of floral bud showed moderate activity of peroxidase. The cytoplasm of epidermal cells and middle layer cells, cytoplasm of pollen grains and stigma cells, cortical cells of style at stage 9 and 12 of floral bud, the nuclei of embryo-sac of ovule at stage 12 cotyledons and seed coat showed little activity.

The acid phosphatase revealed its significant activity in the epidermal cells of anther, tapetal cells, the outer wall of pollen and their nuclei at stage 9 of floral bud. The exine of pollen and their nuclei, outer wall of stigmatic cells and their nuclei, the nuclei of embryosac at stage 12 of floral bud and endosperm showed prominent distribution of acid phosphatase. The epidermal cells of anther, middle layer cells, cytoplasm of pollen grains, outer wall of stigma, at stage 12 of floral bud; cytoplasm of pollen tetrad, transmitting tissue, integument and nucellus at stages 9 and 12 of floral bud revealed moderate activity. The cytoplasm of epidermal cells and middle layer cells, cytoplasm of stigmatic cells at stages 9 and 12 and developing seed coat showed poor activity of acid phosphatase.

The findings using polyacrylamide gel electrophoresis revealed the following: The bands of the zymograms of total proteins appeared both as narrow and thick on the particular gel-profile of reproductive and vegetative parts of potato plant.

There occurred three bands in the zymogram of pollen grains, two bands each in the style and true seeds. However, a single band appeared in the gel-profile of each of the anther, stigma and ovary.

Three and two bands appeared on the gel profiles of underground and
aerial tubers respectively.

The gel-profiles of both the glycoproteins and lipoproteins of reproductive organs such as anther, pollen, stigma, style, ovary and seeds; floral parts such as calyx and corolla and vegetative organs such as underground and aerial tubers revealed merely a single band each. However, the bands formed by glycoproteins were more in thickness than those formed by lipoproteins. In general the gel profiles of nucleic acids occurred as thick bands.

The zymograms of nucleic acids of anther pollen, style, stigma and ovary revealed three bands followed by two bands in the gel-profiles of stigma and seeds.

The zymograms of underground tuber and aerial tuber extracts showed two and three bands respectively.

The maximum number of isozyme bands of peroxidase were revealed on gel-profiles of true seeds and pollen grains as enzyme five. Four bands of isozymes were observed both in ovary and styler tissue. Three bands of isozymes appeared in the gel profile of peroxidase of anther and only two bands were revealed in the zymogram of stigma.

The underground potato tuber and aerial tuber revealed four bands each in their zymograms for peroxidase.

Amongst the reproductive organs the seeds revealed maximum number of catalase isozyme bands being five, closely followed by three isozyme bands each of stigma, pollen grains and ovary. Least number of these bands appeared in the gel profiles of style and anther tissue extracts as two.

In the vegetative organs four bands appeared in the zymogram of underground tuber and two bands were revealed by aerial tuber extract. Narrow isozyme bands appeared in the gel profile of the enzyme catalase for reproductive and vegetative organs of potato plant.

The enzyme tyrosinase revealed maximum activity in true potato seeds with the number of isozyme bands formed as three. Two bands were observed each in pollen, style, stigma and ovary. Anther revealed a single
isozyme band in its zymogram.

The underground tuber and aerial tuber extracts revealed the appearance of two bands.

The present work will serve useful to many botanists engaged in biochemical and histochemical work that will also add to the current literature of its kind. It will equally have important practical application. The findings reported in this thesis are original and have not been submitted for the award of any degree of this or any other university.

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