DISCUSSION

This study was undertaken with a view to investigate the effect of phenolics and GA₃ in relation to photoperiod on growth and development of two plants, *Panicum miliaceum* which is a short day and 'I 22' *Triticale* which is long day in response to photoperiod. The results are discussed here in the light of recent literature available on the subject.

**GA₃ effects growth and development**

The results presented in this thesis demonstrate that GA₃ increases shoot elongation and also hastens
the emergence of ears on the main axis. The hastening of flowering by GA3 has also been reported in quantitative short day plants *Cosmos bipinnatus* (Witwer and Bukovac, 1958; Melder and Owens, 1973, 1974), *Cannabis* (Razumov, 1960) and *Sesaria italica* (Nanda et al., 1977). In fact it has been shown more recently that GA3 even induces flowering in the qualitative short day plants *Chrysanthemum morifolium* (Barbat and Ochesanu, 1964; Pharis, 1972), *Zinnia elegans* (Sawhney and Sawhney, 1976), *Dahlia pinnata* (Kumar et al., 1976), *Impatiens balsamina* L. (Nanda et al., 1967, 1969) under non-inductive photoperiod, while the hastening effect of GA3 on flowering of Triticale is in accord with the reports on many long day plants, that on *Panicum miliaceum* runs contrary to the 'gibberellin-anthesin' concept which has been put forth by Chailakyan (1968) to explain the photoperiodic control of flowering. The effect of GA3 on growth and development, however, varies with the photoperiod and the time of sowing.

**Phenolics enhance growth**

The enhancement in shoot elongation in both *Panicum miliaceum* and 'T 22' Triticale by all the tried phenols reported in this thesis is in conformity with
the results reported earlier from this laboratory on *Impatiens balsamina* L. (Nanda et al., 1976; Nanda and Kumar, 1977; Kumar et al., 1978; Sood, 1976; Sood and Nanda, 1979; Kumar, 1979), on *Avena* (Henderson and Nitsch, 1962) and rice (Bau et al., 1974). Phenols, thus, resemble gibberellins which are known to increase shoot elongation (Nanda, Grover and Chinoy, 1957a; Nanda, 1955; Brian et al., 1959; Wellensiek, 1960; Kamate and Kishimoto, 1960; Osborne, 1965; Michniewicz and Krissel, 1972; Bansal, 1979). Apart from shoot elongation, phenols also increase the number of branches/tillers in both the plants. These results are in accord with the results of other workers on other plants (Vendrig and Buffel, 1961; Henderson and Nitsch, 1962; Ojehon et al., 1968; Miminoshvilli et al., 1973; Michniewicz and Galoch, 1974; Kefeli, 1974; Frederick, 1974; Macháčkova and Zmählal, 1976; Kefeli and Kutacek, 1977; Nanda et al., 1977; Datta et al., 1977; Datta and Nanda 1978) but are contrary to Marigo and Boudet (1975) who reported that exogenous application of phenolics decreases vegetative growth in *Lycopersicon esculentum* Mill. GA3 is also reported to increase petiole length and leaf area in *Trifolium repens* (Fletcher and Martin, 1962), growth in barley and wheat (Large et al., 1951;
Radley, 1970 and Kirby, 1972) and tillering in many grasses (Leopold, 1949; Kraus, 1954; Brian and Hemming, 1955; Brian et al., 1958).

Phenols hasten ear emergence

Another point in which phenols resemble $GA_3$ is the hastening of ear emergence in both these plants. Phenols have been reported to hasten floral bud initiation in Impatiens balsamina L. (Nanda et al., 1976; Nanda and Kumar, 1977; Kumar et al., 1978; Kumar, 1979). The induction of floral buds by SA in *Lehna gibba* $G_3$, a long day plant under non-inductive conditions, has also been reported by Oota (1975). A flower inducing factor from honey dew has been identified as salicylic acid by Cleland and Ajami, (1974) and Cleland (1977). Zucker et al., (1965) also reported that the level of chlorogenic acid increased in the leaves of both the long day and short day species of Nicotiana and also the fronds of *Lehna gibba* $G_3$ (Isemoto, 1971) under inductive conditions. Lee and Skoog (1965a, b), Tomaszewski, and Thimmann (1966) reported that salicylic acid and 4-hydroxybenzoic acid stimulate bud formation in tobacco cultures. More recently, Pieterse (1976), Pieterse and Muller (1977) and Khurana and Maheshwari (1978) have shown that salicylic acid is
able to induce floral buds in *Lemna gibba* G3 and duck weeds under non-inductive photoperiods. These results are, however, contrary to Pryce (1972) who reported that gallic acid, a polyphenol acted as a natural inhibitor of flowering in *Kalanche blossfeldiana*.

The effect of photoperiod on flowering of these two plants is as expected. Thus, in *P. miliaceum* which is a quantitative short day plant ear emergence is hastened by SDs but in triticale which is a quantitative long day plant it is hastened by LDs.

**Biological activity of phenolics is not related to molecular configuration**

According to Hess (1966), the presence of two OH groups at ortho position with a free para position is necessary for the biological activity of phenolic compounds (Fig.172). The results presented in this thesis do not support this postulate. Thus, in resorcinol, one OH group is located at para position and the other at meta position. Yet, it increases grain yield more than catechol; and pyrogallol, in general, more than chlorogluicinol. Again G1, which has the OH group at the ortho position also affected ear emergence to the same extent, although it did not induce floral buds as such and, in fact, delayed the G3- caused induction of
floral buds in Impatiens balsamina. On the other hand, the para-substituted ortho diphenols CA and DOPA, did induce floral buds, the latter with an amino acid substitution being more effective than the former with a carboxylic acid substitution (Kumar, 1979). It would, thus, appear that the affect of phenolic compounds on growth and development is independent of structural configuration. Gortner and Kent (1956), Gortner (1962), Basu (1962, 1972), Kefeli and Kutacek, (1968, 1970) and Dhawan et al., (1976) also found no relationship between the structure of a phenol and its activity nor do they support the concept that monophenols inhibit growth and differentiation while di- and polyphenols promote it (Nitsch and Nitsch, 1962; Pilet, 1964, 1966; Aberg and Johanson, 1969).

Phenols increase ear length and number and weight of grains

It is significant that phenols in general increase ear length, number of ears per plant, number and weight of grains per ear and the thousand grain weight— all the characters which determine the ultimate economic yield. Similar results have been reported by Nanda et al., (1977) and Datta et al., (1977) in Setaria italica and
other millets. It would, thus, appear that these phenols cause morphogenetic effects of great significance. This is probably due to the enhancement of growth and increased productive branches and tillers, which are expected to produce longer ears and more grains due to an increased photosynthetic produced by increased number and size of leaves. It would appear that phenols play a significant role in mobilizing food reserves for translocation to the ears for grain filling process. This is supported by the fact that phenols increase the activity of hydrolyzing enzyme for grain filling process (sinks) as is also demonstrated earlier by Kumar (1979) in Impatiens balsamina L. This point will be discussed further while dealing with the effect of these phenols on changes in the activity and electrophoretic pattern of enzymes. The effect of GA₃ on ear length, grain number, thousand grain weight and ultimate yield depends upon its concentration. Thus, while lower concentrations are stimulatory, higher concentrations exert an inhibitory effect. Pillai and Chandhok (1961) also reported that lower concentration of gibberellic acid increased ear length, spikelets, number of grains and grain weight in Sorghum vulgare. Soni and Yousif (1976) reported that GA₃ improved fruit setting in apricot.
Synergistic effect of GA$_3$ and phenols

The most interesting point that has emerged from this investigation is the synergism between phenols and GA$_3$. Synergism between IAA and phenols in their effect on some growth and differentiation processes has been reported by a number of workers (Rabin and Klein, 1957; Henderson and Nitsch, 1962; Nitsch and Nitsch, 1962; Thimmann et al., 1962; Tomaszewski, 1964; Tomaszewski and Thimmann, 1966; Fadi and Hartmann, 1967; Czerner, 1969; Basu, 1969, 1970, 1972; Roy et al., 1972; Basu et al., 1973). But synergism between phenols and GA$_3$ in flowering of *Impatiens balsamina* was reported from this laboratory for the first time, (Nanda et al., 1976; Nanda and Kumar, 1977 and Kumar et al., 1978; Sood and Nanda, 1979; Kumar, 1979) and is supported by the results on ear emergence reported in this thesis.

Effect of phenols changes with photoperiod

It is rather interesting that while *Panicum miliaceum* and *Triticale* belong to two different photoperiodic response types and while in the former flowering is hastened, in the latter it is delayed by short days, the number and weight of grains produced
in both cases is higher under ND than under SD or LD conditions. These results clearly demonstrate that while the photoperiodic requirement for the change of the growing apex from vegetative to the reproductive state differs, requirement for the completion of gametogenesis and embryogenesis in the two is similar. The photic requirement of light and temperature conditions has been studied extensively in oat (Tsayal and Nanda, 1962), *Cicer* *arilletinum* (Nanda and Chinoy, 1960), *Oryza* species (Butany and Sampath, 1960), *Panicum miliaceum* (Nanda, 1958), wheat (Chinoy and Nanda, 1951, 1952; Riddell et al., 1955; Miera, 1953; Nanda et al., 1959) *Panicum miliaceum* and *Setaria italicita* (Nanda, Grover, and Chinoy, 1957, 1957a) barley (Johnson and Taylor, 1955), *Eschscholzia californica* (1976); *Papaver rhoas L.* (Nanda, 1961); cotton (Bhargava and Bhardwaj, 1969), and other plants (Borthwick and Parker, 1938, 1939; Parker and Borthwick, 1939; Borthwick et al., 1941; Borthwick et al., 1948; Schmitz, 1951; Downs et al., 1958, 1959; Ketelapper, 1965; Sinha et al., 1973). More recently, Nanda et al., (1976) demonstrated that *Setaria italicita* behaves as a quantitative short day plant in May, July and September but as a qualitative short day plant in November sowing.
The interaction of photoperiod and temperature in the flowering response of plants has been demonstrated by other workers as well (Ogawa, 1960; Hackett and Hartmann, 1967; Rünger, 1968; Moe and Wicker, 1973).

Sowing time affects growth and development

That light and temperature conditions affect growth and development of plants is also evident from the fact that plant height, number of branches, days taken to ear emergence, number and length of ears and number and weight of grains changes with the time of sowing. Thus, while in experiments 1-4 ears were produced on all plants under all the three photoperiods, in experiment 5 ears were not formed at all. Again in triticale, the number and weight of grains was higher in experiment 7 than in experiment 8. Seasonal effects on growth and development of lettuce, oat, pea, oil seed rape, sugar beet and spring cereals and beans have also been reported by many workers (Griffiths, 1961; Proctor, 1963; Milbourne and Hardwick, 1968; Hull and Webb, 1970; Jessop and Ivins, 1970; Scott et al., 1973; Gray, 1976; Gray and Morris, 1978). Ojehomon et al. (1968) reported that long photoperiods caused abscission of flowers.
and buds on the main axis and decreased the number of flowers, seeds and pods/plant in *Phaseolus vulgaris*. Similar studies were also carried in other economic plants by other workers (Sen Gupta and Sen, 1944; 1952; Bhargava and Sharda, 1969; Rao et al., 1972; Singh and Choubey, 1972; Bose, 1974; Kamlesh, 1977).

**Phenols and GA₃ change the pattern of isoperoxidases**

The appearance of new isoperoxidases in the stem as well as in the leaves under both inductive and non-inductive conditions clearly indicates that the pattern of isoperoxidases changes with the developmental stage of the plant. Warner and Upadhya (1968) also observed differences in the number and activity of isoenzymes of esterases, leucineaminopeptidases, peroxidases and amylases in plants receiving short day and long day treatments. McCune (1961), Galston and McCune (1961), Ockerse et al., (1966) and Sawhney et al., (1979) have also shown that GA₃ induces both quantitative and qualitative changes in electrophoretic pattern of peroxidases. Lee (1972) considered that it was the relative level of isoenzymes rather than the total activity of peroxidase which was related to differences
in growth and development of plants. Nanda et al., (1973),
Nanda and Dhaliwal (1973) and Gurumurti and Nanda (1974)
showed that isoperoxidases concerned in the initiation
of roots were different from those concerned in their
development. Tao and Khan (1976) showed that the number
of peroxidases and the activity of certain enzymes
increased with the increasing period of stratification of
pear embryos. That the peroxidases cause the oxidation of
endogenous phenoles has been shown by a number of workers
(Jones et al., 1970; Pickering et al., 1973; Reight et al.,

Peroxidases are involved with respiration
(Lundegardh, 1954). The new isoperoxidases developed
under inductive conditions may increase respiratory
activity and thereby increase oxidation of reserve food
materials and their mobilization to the apex for flowering.
Sawhney et al., (1972) have also shown that the activity
of both amylases and catalases was enhanced when
Impatiens balsamina was transferred to inductive
photoperiods.

The fact that more isoperoxidases developed in
the leaves of plants exposed to inductive photoperiods
than in those treated with GA3 or SA each alone or
together under LD condition lend support to postulate put forth earlier (Tewari, 1975) that induction caused by short days is probably stronger than the GA$_3$- or phenolic-cased induction under non-inductive photoperiods. Goren and Tomer (1971) reported that phenols like seselin and coumarin, affect growth, indoleacetic acid oxidase and peroxidase activity in cucumber (*Cucumis sativa* L.) radicles. Recently Rao et al., (1976) have found increased activities of peroxidase and amylase in the developing grains of triticale, wheat and rye plants.

*Phenols and GA$_3$ affect PPO activity*

The increase in polyphenol oxidase activity in both the stem and the leaves of plants treated with SA, GA$_3$ and SA + GA$_3$ under both photoperiods is probably due to the appearance of some new isoenzymes. An increase in polyphenol oxidase activity in the developing tissue has also been reported by Srivastava and Huystee, (1973); Stahmann et al., (1966); Stafford and Galston (1970); and Taneja and Sachar (1974).

The decrease in polyphenol oxidase activity subsequent to floral development reported in experiment 6 is identical to a decrease during the maturation of kernel subsequent to an increase during kernel development.
More recently, Habaguchi (1977) showed that the regulation of polyphenol oxidase activity was closely related to physiological changes in the cells. Leopold and Plummer (1961) reported that polyphenol oxidase catalyzed the condensation of phenols with IAA. According to Noyed and Tuli (1966), PPO possibly synthesizes the molecular 'key' that unlocks the biochemical apparatus of dedifferentiation concomitant with primordium initiation.

Phenols and GA₃ affect amylase activity

Apart from peroxidases and polyphenol oxidases, some new isoamylases also develop in the stem as well as in the leaf under both photoperiods. Nanda et al., (1969) have shown that while both GA₃ and GA₁₃ induced floral buds, it is only GA₃ which enhances stem elongation. It is, thus, apparent that while increase in amylase activity may be related to enhanced stem elongation, it may not be concerned in the initiation of ears. It is known that carbohydrate supply is related to increased extension growth that accompanies the transformation of the meristem from vegetative to the reproductive state (Chinoy and Nanda, 1951, 1956, 1961; Nanda et al., 1957;
Thomas, 1961; Tayal and Nanda, 1962; Nanda and Krishnamoorthy, 1967; Clines and Agatep, 1970; Sharma and Nanda, 1976). Of course, it is difficult to say whether hastening in ear emergence is caused directly by an increase in amylase activity or is an indirect effect caused by increased extension growth which accompanies the inductive process. As treatment of plants with SA does not increase amylase activity of the stem under non-inductive photoperiods significantly, even though it induces ear emergence, it may be assumed that amylase activity is not directly involved in the inductive process. This assumption is supported by the fact that the GA$_3$- or phenolic caused in the turn over or synthesis of amylase is rather short lived, as enhanced rate of amylase activity is followed by a steep fall. This decrease in the activity may be due to exhaustion of food materials in increased extension growth. These results are contrary to Broda (1966) who reported that coumarin and caffeic acid act as inhibitors of $\alpha$- and $\beta$-amylases and Chrispeels and Varner (1966) who reported that inhibition of gibberellic acid induced formation of amylase by abscein.

The increase in grain yield of *Panicum miliaceum*
and triticale reported in this thesis is probably due to the enhanced activity of hydrolyzing enzymes, as has also been reported by others as well (Paleg, 1960; Yomo, 1967; Varner, 1964; Nanda and Purohit, 1965; Nanda and Dhindia, 1966; Sawhney et al., 1970). Johri and Maheshwari (1966) also reported that α- and β-amylase activity increased in Opium Poppy before cell wall begins to be laid in the endosperm and then when the cotyledons elongate. More recently it has been reported that increase in α- amylase, carbohydrate content and peroxidases are related with the kernel development in triticale, wheat and rye plants (Rao et al., 1976; Klassen et al., 1979).

Phenols and GA₃ cause changes in RNA

The increase in content of RNA both in the stem and the leaves during the earlier stages and the enhanced transcriptional activity reported in experiment 6 is very much expected to meet the enhanced demand for protein synthesis for meristematic cells to participate in floral induction and subsequent morphogenesis (Healey and Jensen, 1965; Suge and Osada, 1967; Gressel et al., 1970; Evans, 1971; Vince Prue, 1975;
Seidlova, 1976; Seidlova and Krekule, 1977; Sawhney, 1976). But the more interesting point that emerges from these results is the induction of new RNA species in stem (Rms 0.21, 0.33 under LD and Rms 0.40, 0.86 under SD) as well as in the leaf (Rms 0.32, 0.36, 0.58 under LD and Rms 0.73, and 0.77 under SD) which is almost always associated with floral bud initiation in both long and short day plants (Nanda et al., 1957a; Nanda, 1958; Chinoy and Nanda, 1960; Nanda and Chinoy, 1960; Nanda and Krishnamoorthy, 1967; Sharma et al., 1978).

GA$_3$-caused synthesis of new RNAs in barley aleurone layer has been reported by Jacobson and Zwar (1974). Evidences are now available to show that nucleic acid metabolism is involved in floral bud initiation (Heishop-Harrison, 1960; Butenko and Chailaknyan, 1961; Zeevaart, 1962; Lang, 1965; Knypi, 1965; Bernier, 1966; Chinoy, 1967; Evans, 1971; Stiles and Davies, 1976; Wardell, 1976; Sharma et al., 1978).

New RNAs associated with growth and development

The development of a new RNA band in plants maintained either under inductive photoperiods or treated with GA$_3$ or SA under non-inductive photoperiods,
indicates that inductive treatments cause 'de novo' synthesis of this RNA band which is associated with the initiation of ears on the main axis. Similar qualitative differences in the RNA band patterns under inductive and non-inductive conditions have been reported by Watson and Mathews (1966) in *Chenopodium amaranticolor* by Yoshida et al. (1967) in *Pharktie nil*, by Konli (1976) in *Amaranthus* and Sharma et al., (1976) in *L. halsemae*. The appearance of these bands in the stem inspite of the fact that it does not bring about photoperiodic stimulus, is rather interesting. As this band developed earlier in the leaves than in the stem it may be considered that this band is actually synthesized in the leaves and is then transported to stem for causing a change in the axillary meristems from vegetative to the reproductive state. But as no evidence is available in literature to show the transportation of macromolecules from one organ to the other, it may be assumed that the synthesis of this RNA band was localized. Apart from this, the development of some other bands in the stem but not in the leaves under both photoperiods appears to be associated with process(es)
other than ear emergence such as enhanced extension growth and increased number of branches. Saikova and Platonova (1966) and Emanuel (1968) have emphasized that phenolic compounds play a significant role in various biological functions. Degreesh (1964) observed that coumarin and trans-cinnamic acid affected DNA content and growth of pea roots.

**Conclusion**

It would appear from the foregoing discussion that nucleic acids and protein metabolism are involved in the change of the growing apex from vegetative to reproductive state either by an increasing the activity or by changing the electrophoretic pattern of the concerned enzymes. Thus, isoenzymes of oxido-reductases as well as of hydrolytic enzymes are associated with developmental phases; some of these are involved in the inductive process, others in the development of induced ears and still others in growth. The synthesis and/or increased activity of the new isoenzymes in many cases is at the cost of turn over of others, so that the total activity of the enzyme as such does not change. In other words new isoenzymes are better adapted to deliver the right goods at the right time.
The induction of earing by phenolics under non-inductive photoperiods is rather interesting particularly their synergism with gibberellic acid in hastening ear emergence and in increasing the number of branches and ears. These results are particularly significant as phenolic compounds increased the number of grains, grain weight and the 1,000-grain weight hence the ultimate grain yield in both *Panicum miliaceum* and *Triticale*.

While the exact mechanism of action by phenols on these morphogenetic phenomena still remaining elusive, the results presented in this thesis demonstrate that phenols like GA₃, realize their biological potency through induction and/or enhancement in the rate of synthesis of specific enzymes and RNAs. While more work is needed to understand the mechanism of action of these phenolic compounds, their potential in increased productivity cannot be doubted in the light of the results presented in this thesis.