Pak. J. Bot., 13(1): 45-48, 1981.

GERMINATION AND GROWTH INHIBITORS IN THE FRUITS OF *PITTOSPORUM TOBIRA* AIT., DURING RIPENING.

M. ISHAQ KHAN* AND M.A. MOKAHEL

Department of Botany, Faculty of Science, University of Gharyounis, P.O. Box 9480, Benghazi, Libya.

Abstract

Germination and growth inhibitor (s) in different fractions of ripening fruits of *Pittosporum tobira* was studied. Both unripe and ripe fruits contained germination and growth inhibiting substance (s) in the water-soluble, acidic and neutral fractions. As compared to unripe fruits, germination and growth inhibitor (s) increased 2-3 folds in the ripe fruits. It is suggested that the increase in acidic inhibitor (s) during ripening may reflect the increase in the level of endogenous ABA-like substances.

Introduction

Fruit ripening involves biochemical and structural events associated with the decline of astringent material such as phenols (Reeve, 1959), disappearance of chlorophylls and a marked increase in ethylene production (Crane, 1964). Burg (1963) observed that separation of fruits from the trees promotes ripening suggesting that the signal for ripening may originate from inside the fruit. Varga & Koves (1959) found that the growth inhibitor (β - inhibitor complex) of a number of fruits increased progressively with the increase in maturation of fruits. They also observed that in maturing stems and fruits the inhibitor accumulated from the ageing leaves. Goren & Goldschmidt (1970) also found an increasing quantity of β-inhibitors in the water phase after solvent partition of ripening citrus fruits. No systematic work seems to have been carried out on the quantitative changes of the inhibitors of germination and growth in ripening fruits. The experiments described in this paper were conducted to obtain information regarding the level of acidic, neutral and water-soluble inhibitors of germination and growth during ripening of the fruits of *Pittosporum tobira* Ait.

Materials and Methods

Unripe hard and ripe soft fruits of *P. tobira* were collected in February 1979 from one single plant growing near the Faculty of Science of the University of Gharyounis, Benghazi, Libya. Unripe and ripe fruits, 100 g portions of each, were extracted separately

*Present Address: Department of Botany, University of Karachi, Karachi-32, Pakistan

with 80% methanol at 7°C in dark for 48 h with three changes of alchool, using 5 ml per gram fresh weight each time. After filteration, methanol was evaporated at 45°C in a forced-air oven and the aqueous layer extracted three times with ethyl acetate. Aqueous and organic layers were further separated and used for isolation of the inhibitor (s). Aqueous fraction was shaken with activated charcal @ 1 g per 10 g fresh weight of fruits and filtered. Charcoal was washed five times with distilled water and extracted with three changes of acetone. Acetone was evaporated to dryness and redissolved in 80% methanol to give 10 g per ml water-soluble fraction (W). Organic ethyl acetate fraction was shaken with 200 ml of 2% $NaHCO_3$ solution and the ethyl acetate fraction was washed twice with water, dried over anhydrous sodium sulphate,evaporated to dryness and redissolved in 80% methanol to give 10 g per ml of neutral fraction (N). The remaining aqueous layer after extraction with ethyl acetate was adjusted to pH 2.5 with 0.5 N HCl, extracted with ethyl acetate several times, dried and evaporated to dryness and redissolved in 80% methanol to give 10 g per ml of acidic fraction (A).

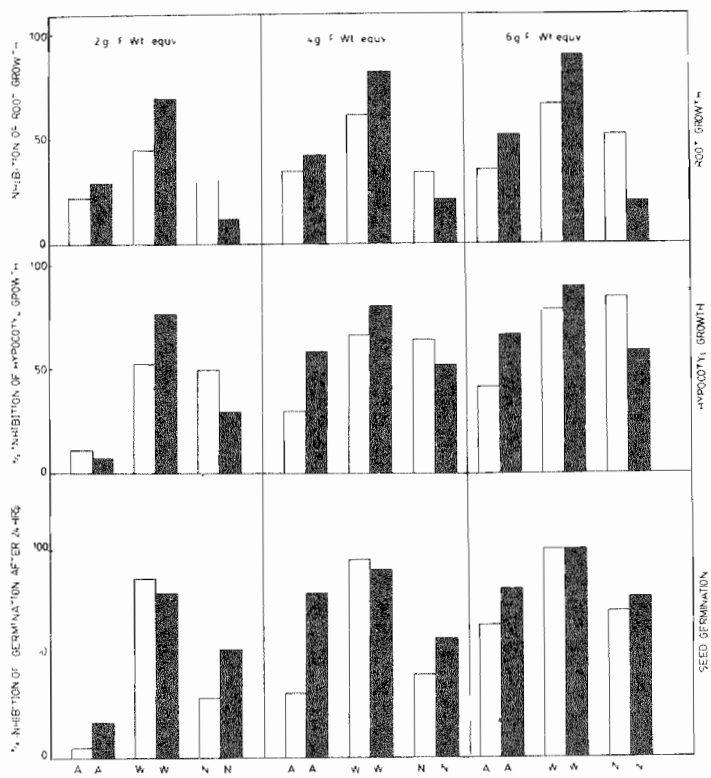

Fig. 1. Effect of water-soluble (W), acidic (A) and neutral (N) fractions from unripe (White columns) and ripe (Black columns) fruits of *Pittosporum tobira* on the % inhibition of germination and growth of *Lactuca sativa* (cv. Great Lakes). Each value is an average of three replicates.

The acidic, neutral and water-soluble fractions of unripe and ripe fruits of 2, 4 and 6 g fresh weight equivalent were poured onto one sheet of Whatman No.1 paper kept in 9 cm Petri dishes together with 1 ml of distilled water. Extracts were evaporated to dryness with the help of hair drier. Later 2 ml of distilled water was added and after standing for 24 h at room temperature (24 ± 1°C), 50 seeds or 15 seedlings of *Lactuca sativa* cv. Great Lakes were placed on each dish and kept at 24° ± 1°C in complete darkness. Seedling used were grown in dark for 48 h. Germination and growth were recorded in diffuse day light.

Results and Discussion

Unripe and ripe fruits of *P. tobira* contained large quantity of germination inhibiting substance(s) as shown by their ability to inhibit the germination of lettuce seeds (Fig. 1). Of all the three fractions, water-soluble fraction of both unripe and ripe fruit showed the highest inhibiting ability of seed germination. As compared to unripe fruits, the germination inhibitor(s) increased substantially in acidic and neutral fractions of the ripe fruits, while the inhibitors present in the water-soluble fraction decreased in ripe fruits or showed no change. During the process of ripening, germination inhibiting ability of the acidic fraction of ripe fruits increased 2-3 fold. This would suggest that the increased germination inhibiting ability of ripe fruits is to control the germination of seeds especially when they are within the ripe fruits. Junttila (1976) also found the appearance of germination inhibitors of lettuce seeds in the acidic ethyl acetate and water-soluble fractions of mature fruits of *Beta vulgaris*. Whereas the methanolic extract did not inhibit the germination of red beet seeds while the water fraction did so.

Similar to germination inhibitor(s), unripe and ripe fruits contained large quantity of growth inhibiting substances in all the three fractions (Fig. 1). Water-soluble and acidic fractions of ripe fruits decreased the growth of lettuce hypocotyls and roots more than those of unripe fruits. Unlike germination inhibitors, the level of growth inhibiting substance(s) of neutral fraction in ripe fruits was less than unripe fruits. Goren & Goldschmidt (1970) also found a substantial amount of "β-inhibitor" in orange fruits approaching ripening to be present in the water phase after solvent partition.

The process of fruit ripening is characterized by a number of biochemical changes such as the conversion of pectins into pectic acids, hydrolysis of polymeric carbohydrates to oligo- and monosaccharides, development of flavoring substances, degradation of chlorophylls and the rise in the production of ethylene which initiates a chain of biochemical reactions that finally leads to fruit ripening (Hess, 1975). Mayak & Halevy (1972) found that a rise in the production of ethylene in senescing aged petals of rose resulted in the increase in abscisic acid-like growth inhibitors. Goldschmidt *et al* (1970) also found a substantial increase in ABA-like growth inhibitor present in the acidic diisopropyl ether

fraction of ripening and senescing fruits of citrus. THe rise in the level of acidic inhibitor (s) of germination and growth in the ripening fruits of *P. tobira* observed in the present study might suggest the possible increase in ABA-like inhibitors of germination and growth during fruit ripening.

References

Burg, S.P. 1963. Studies on the formation and function of ethylene gas in plant tissues. In Regulateurs Naturels de la Croissance Vegetale, J. P. Nitsch (ed.) pp. 719-725. CNRS, Paris.

Crane, J. C. 1964. Growth substances in fruit setting and development. Ann. Rev. Pl. Physiol., 15: 303-326.

Goren, R. and E.E. Goldschmidt, 1970. Regulative systems in the developing fruit. I. The hormonal balance in orange fruit tissues. Physiol. Plant. 23: 937-947.

Goldschmidt, E.E., S.K. Eilati, and R. Goren, . 1970. Increase in ABA-like inhibitors and decrease in Gibberellin-like substances during ripening and senescence of citrus fruits. In Plant growth substances. J. Carr (ed.) pp. 611-617, Springer-Verlag, Berlin.

Hess, D. 1975. Plant physiology, molecular, biochemical and physiological fundamentals of metabolism and development. pp. 249, Springer-Verlag, Berlin, new York.

Junttila, O. 1976. Germination inhibitors in fruit extracts of red beet *(Beta vulgaris* cv. Rubra). J. Exp. Bot., 27: 827-836.

Mayak, S. and A.H. Halevy. 1976. Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. Pl. Physiol., 50: 341-346.

Reeve, R. M. 1959. Histological and histo-chemical changes in developing and ripening pears. I. The catechol tannins. Am. J. Bot., 46: 210-217.

Varga, M. and E. Koves. 1959. Distribution and quantitative changes in the β-inhibitior in the various organs of the bean plant during ontogeny. Acta Biol. 9 ; 369-378.