Acta Bot. Neerl. 26(2), April 1977, p. 183–186.

BRIEF COMMUNICATIONS

POSSIBLE MEMBRANE-ASSOCIATED EFFECTS IN GIBBERELLIC ACID AND PHENYLALANINE-INDUCED ROSE COLORATION ENHANCEMENT

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SUMMARY

When combined with GA, phenylalanine produced marked enhancement of red colouration of rose (*cv* Baccara) petals, this effect significantly exceeding that of GA applied alone, while phenylalanine when administered alone was virtually ineffective in this respect. Parallel assays of phenyl-ammonialyase (PAL) activity indicated essentially similar endogenous levels of enzyme irrespective of treatment. These results are interpreted as indicating that mode of GA action in this system is probably not by direct PAL activation but rather via GA-induced enhancement of membrane permeability thus providing more phenylalanine precursor for anthocyanidin pigment synthesis. This hypothesis is substantiated by the results of plasmolysis studies executed on rose petal epidermal strips in hypertonic solutions where GA significantly cut down time required to achieve a given degree of plasymolysis.

1. INTRODUCTION

A possible mode of GA action has been associated with the plasma membrane (PALEG, WOOD & SAWNHEY 1972, WOOD & PALEG 1972, LESHEM et al. 1976). A mode of GA action not necessarily exclusive of the former has been associated with hormone-induced enzyme activation (LESHEM 1973). In red rose cultivars it has been documented that GA has a promotive effect on anthocyanidin pigment formation (ZIESLIN et al. 1974) and it is possible that either of the two above-mentioned modes of action may be involved in this promotion. One possibility is that GA may act on one of the regulatory enzymes in the metabolic pathway leading to pelargonidin and cyanidin synthesis and possibly in particular PAL which in the shikimic acid route participates in the conversion of PA (phenylalanine) to tCA (*trans*-cinnamic acid). A further possibility is that membrane permeability may be effected by the hormone thus promoting pigment synthesis by means of increment of precursor availability.

2. MATERIALS AND METHODS

2.1. Membrane permeability studies

Changes in rates of plasmolysis and/or deplasmolysis of intact plant cells in hypertonic as compared to control solutions have been associated with changes in membrane permeability (STADELMAN & WATTENDORF 1966, FENG 1973,

FENG & UNGER 1972). In the present investigation plasmolysis was effected on upper epidermal sections of rose (*cv* Baccara) petals whose red colour facilitated microscopic observation. The sections were immersed in 0.9 M mannose citrate-phosphate buffered solutions and time required to achieve 50% plasmolysis was noted. Each treatment was applied employing six replicates. Experimentation included deplasmolysis which proceeded normally thus obviating the possibility of membrane damage.

2.2 PAL and pigmentation determinations

Using a microsyringe 5 µl 0.1 M phosphate buffer pH 6.0 containing GA_3, PA or a mixture of both were injected into the hearts of 5 mm ϕ *cv* Baccara rosebud receptables. The latter were harvested a week later and pigment extracted from petals with acid methanol according to the procedure outlined by ZIESLIN et al. (1974). PAL enzyme activity was determined by acetone extraction of petals at 20 °C and thereafter assayed by the method of RIOV, MONSELISE and KAHN (1969). This method is based upon the conversion of L-PA substrate to tCA and the subsequent spectrophotometric determination of the latter at 290 nm. All assays were carried out in quadruplicate.

3. RESULTS

Table 1 presents reults of the permeability studies. From this table it is apparent that especially at the lower pH values permeability is markedly affected by

Table 1. Effect of GA_3 upon plasmolysis of rose petal epidermal cells in buffered 0.9 M mannose solutions. Figures are 6 replicate means and represent time (mins. and secs.) required to achieve 50% plasmolysis.

pH	Buffer alone	Buffer + GA_3 5 ppm
3.5	1′ 18″	38″
4.5	1′ 14″	41″
5.5	59″	47″
6.5	47″	51″

Table 2. Pigmentation and phenyl-ammonia-lyase activity as effected by application of GA_3 and phenylalanine. Figures presented are means of 6 replicates.

Serial No.	Treatment	Colouration*	PAL activity*
1	Buffer alone	510	2620
2	GA_3 500 ppm	1375	2675
3	Phenylalanine 5 µg/cc	750	2730
4	2 + 3	2195	2730

* O.D. 525 nm.
** O.D. 290 nm.

GA_3 treatment, this effect being diminished when approaching neutral. The normal pH of rose petals at the experimental stages investigated in this research is acidic c. pH 5.0.

Table 2 summarizes effects of microinjection of GA_3, PA or a combination of both upon pigmentation and enzyme (PAL) activity. These results indicate that PA alone had very little effect upon colouration. However, when combined with GA_3 it produced more intense colouration than GA_3 applied alone (O.D. 2195 as against O.D. 1375). The results in *table 2* furthermore show that despite the marked effects on pigmentation, PAL activity manifested very little change.

4. DISCUSSION

The experimental data (*tables 1* and *2*) possibly imply that increased pigmentation produced by the application of GA either alone or in combination with PA is not a result of PAL activation since this enzyme's activity remained essentially constant irrespective of treatment. The pigmentation increment may alternatively be accounted for by GA_3-enhanced permeability of membranes and concomittantly, an increase of anthocyanidin precursor in the form of PA. The ability of GA to significantly enhance permeability is indicated by results of the plasmolysis experiments (*table 1*). This hypothesis may furthermore be borne out by the observation (*table 2*) that PA, when applied alone, has only little effect but when combined with GA the pigmentation exceeds that of GA applied alone.

ACKNOWLEDGEMENT

The authors wish to thank Mrs. Jeanette Barnes for her able technical aid.

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