FACTOR(S) REMAIN IN ETHYL ACETATE EXTRACT SYNERGISTICALLY ACT WITH ABSCISIC ACID IN INDUCING WOUND PERIDERM FORMATION OF POTATO TUBER

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ABSTRACT

Little is known about the endogenous factor(s) that involved wound periderm (WP) formation. The aim of this study was to determine the endogenous factor(s) that induce WP formation. Tissue discs were prepared from medullary tissues of potato tubers and the preparation regards to as wounding. The washed tissue discs were treated with different plant hormones, and abscisic acid (ABA) showed a stimulatory effect in inducing WP formation. However, ABA cannot fully recover WP forming ability suggests some unknown compounds might also play a role in the formation of WP. Various organic solvent fractions obtained from cortical tissues of freshly harvested potato tuber were examined in the presence or absence of ABA. Ethyl acetate (EA) fraction in the presence of ABA fully restored WP forming ability of washed tissue disc. Thus the WP forming factor(s) might be present in the EA fraction and synergistically act with ABA.

KEY WORDS: Potato tuber, suberization, wound periderm, abscisic acid.

INTRODUCTION

Wounding of potato tubers is a common phenomenon during harvesting and post harvest handling which can increase water loss and infection by microorganisms. When potato tubers are wounded, a protective layer next to the wound surface was formed (Lulai 2004). The layer is called wound periderm (WP). On the cell wall of this cork-tissue, suberin that consists of phenolic and aliphatic compounds is accumulated. The intact suberized layer of the tuber periderm provides a durable barrier both to the diffusion of water and to the invasion of pathogens. Suberization develops a distinct polyaliphatic layers with associated phenolic acids deposit between the primary cell wall and the plasma membrane prior to secondary wall formation (Ruth et al 1994, Borg-Olivier and Monties 1989, Borg-Olivier and Monties 1993). This multilayered aliphatic polymers with its associated waxes is thought to provide suberized tissue with their water repellent (Lulai and Orr 1994, Bernards and Lewis 1998).

In case of potato, rate and extent of WP formation and suberization are largely dependent on temperature (Perveen et al. 2010). Temperature that favors maximum suberization and periderm formation can also promote tuber ageing, sprouting and most importantly, the growth of microorganisms that may cause decay (Hornbacher et al. 2001). Relative humidity is reported to be important but less critical than temperature in the wound healing process. With low humidity desiccation of the wound surface takes place that inhibits suberization (Perveen et al. 2010). In contrast, saturated atmospheres may hamper WP development by inducing cell proliferation at the wound surface (Lulai 2004, Perveen et al. 2010). Various hormones seemed to be important factors in the signal transduction pathway leading to the process of suberization (Hornbacher et al. 2001). Since suberization appears to be triggered by wounding, it is likely that the hormonal compounds that lead to WP formation are generated soon after the wounding. The loss of WP forming ability was brought about by thorough washing of potato disc (Perveen et al. 2010). Various cytokines are removed by washes and when exogenous cytokines were applied to wash tissues that can partially restore suberin production (Perveen et al, 2010, Robb et al. 1991). Among various cytokines, abscisic acid (ABA) is a critical factor for WP formation. However, ABA stimulated WP formation and suberization process but it couldn't fully restore WP forming ability of tissue discs when compared to unwashed control discs (Perveen et al. 2010). So the presence of any other factor(s) that might act synergistically with ABA to induce WP formation. Further investigation is required to find out the factor(s) other than ABA responsible for WP formation. The present investigation was undertaken using potato plants as material in order to know further on the suberization and WP formation of tissue discs under certain experimental conditions.

Understanding of the basis for suberization in potato tuber could lead to innovative control measure for diseases and dehydration during storage. These important post harvest process will provide effective means to improve the storability and nutritional quality of this important crop.

MATERIALS AND METHODS

Preparations of tissue discs from potato tuber:

Discs were prepared from the Medullary tissue of potato tuber (*Solanum tuberosum* L. cv. Irish Cobbler) using cork borer (2.0 cm in diameter and 5.0 mm in thickness with a fresh weight of approximately 2.0-2.5 gm). For incubation, discs were kept within a Petri dish that contained a small dish filled with water to provide humid conditions. Petri dishes were then incubated at 25°C in the dark for 7 days (own) to induce WP formation (Perveen et al. 2010).

Measurement of WP formation:

Since the WP act as a strong barrier to water diffusion, the resistance of the tissue surface to water diffusion is a good measurement of degree of WP formation. At first, for this purpose we measured water potential of the tissue discs. But the procedure was very time consuming and the result obtained had a large variation. So in this experiment water loss was measured from the tissue discs during the incubation in open space. After the 7 days-incubation in a moist Petri dish, the discs were transferred to open space at 25°C (relative humidity, ca. 30 percent) and time course changes in fresh weight was measured. The percent of initial water content of potato tissue discs was calculated from the following formula-

WC = water content of a tissue disc/Initial water content of tissue disc X 100

Where, water content of a tissue was calculated from the subtraction between the fresh weight at different day interval and dry weight of the tissue disc. Dry weight was measured after drying the tissue discs at 95°C overnight. All experiments were repeated at least twice with 20 replicates.

Preparation of solvent fractions obtained from potato cortical tissues:

In order to detect endogenous factor(s) that induce WP formation, various solvent fractions were prepared from cortical tissues of potato tubers. Peeling of potato tubers that contained cortical tissues was homogenized with ethanol to make final 70% ethanolic extract. The homogenate was filtered through a Buchner funnel. The extract was then evaporated to remove ethanol at 45°C. The concentrated crude extracts were then partitioned three times with an equal volume of ethyl acetate (EA). The EA fraction was then concentrated to dryness. The aqueous phase (Aq) was again partitioned three times with an equal volume of n-Butanol. The butanol fraction (But) was then evaporated to become dried. The remaining aqueous phase was also concentrated to reduce its volume as much as possible. EA and But fractions were then re-dissolved with methanol. All these fractions were kept at -30°C until further use.

Synergistic effects of solvent fractions and ABA on WP formation:

Each EA and aqueous fraction was applied to tissue discs together with different concentrations of ABA (0, $5x10^{-10,}$ $5x10^{-9}$, $5x10^{-8}$, $5x10^{-7}$ mol) to observe their synergistic effect on WP formation.

RESULT AND DISCUSSION

EA fraction of potato extract synergistically act with ABA to induce WP formation of potato tuber:

The complete inhibition of WP formation was brought thorough washing and it was observed that exogenous ABA showed a significant enhancement of WP formation to the washed tissue discs. However, it couldn't fully restore WP forming ability (Perveen et al. 2010). So, to find out the other factor(s) that act

synergistically with ABA for WP formation, ABA was applied on washed tissue discs together with different solvent fractions of potato tuber. It was observed that together with ABA both ethyl acetate (EA) and aqueous (Aq) fractions fully restored the WP formation of washed tissue discs (Fig. 1). This finding suggested that factor(s) might exist both in EA and Aq fraction. The amount of ABA that was exogenously applied to tissue discs was about 1,000 times higher than that of endogenous ABA (data not shown). It is necessary to examine therefore, synergistic effects of the extracts and ABA at lower concentrations. The aqueous fraction showed appreciable WP forming activity in the presence of ABA more than $5x10^{-8}$ mol. On the other hand, the EA fraction showed the activity at low level of ABA ($5x10^{-10}$ mol) (data not shown). The result indicated that the factor(s), which acts as a synergist of ABA in inducing wound periderm, is present in the EA fraction. However, EA and Aq fraction was alone unable to induce WP formation significantly (Fig. 2). So all these results indicated that though ABA is the critical factor for WP formation, it requires other unknown factor(s) to induce WP formation fully.

CONCLUSION

Though ABA is the most important factor for WP formation, but other factor(s) is also required to fully restore it. Understanding of the mechanisms for suberization in potato is very important for increasing shelf life and the quality of this important crop. So, further investigation is required to find out the factors other than ABA responsible for WP formation.

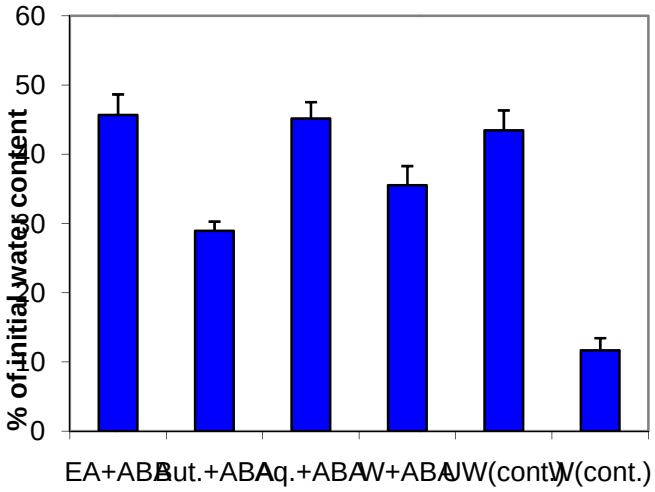

Fig. 1: Synergistic effect of various fractions of potato extract with ABA in wound periderm formation. Bars represent ± SE (n=20). Among the fractions tested EA and Aq fraction together with ABA showed equal WP formation and when compared to unwashed control discs. EA-ethyl acetate fraction; But-butanol fraction; Aq- aqueous fraction; W-washed; UW-unwashed control W (cont.)-washed control; ABA- abscisic acid.

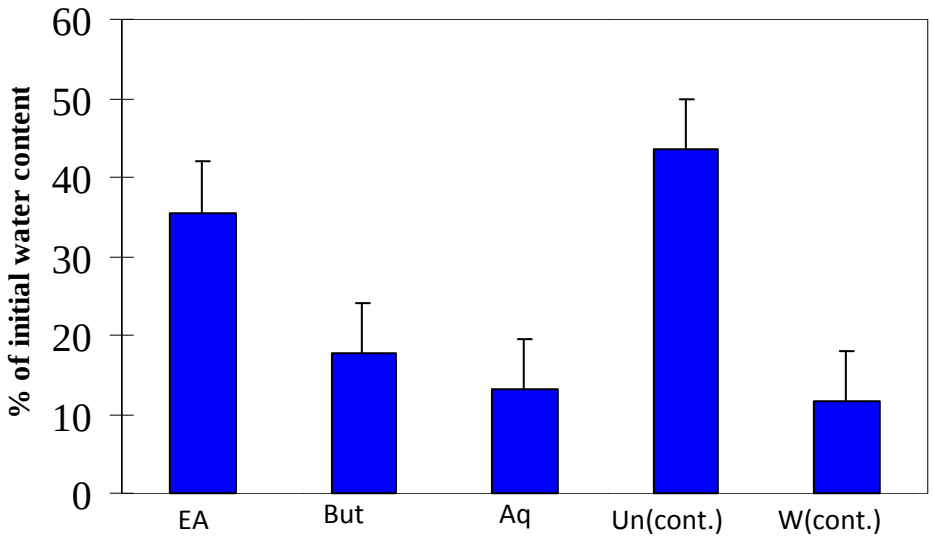

Fig. 2: Effect of various fractions of potato extract in wound periderm formation. Bars represent ± SE (n=20). Among the fractions tested EA fraction showed highest WP formation and when compared to unwashed control discs. EA-ethyl acetate fraction; But-butanol fraction; Aq- aqueous fraction; W-washed; UW-unwashed control W (cont.)-washed control.

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