



ORIGINAL ARTICLE

Physiological effects of CN-resistance Alternative Pathway on Climacteric Respiration of Banana fruit

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ABSTRACT

CN-resistance alternative pathways an effective pathway on the fruit ripening process at the time of connect to the plant and when removed from it, but it needs more investigations. Understanding the influence of this pathway on the fruit ripening led us to an experiment that was carried out as One-Way ANOVA with three replications. The measured traits included: fruit weight loss on the last day, total dissolved solids (brix), and the fruit quality scores on the sixth and last days. The results showed that the respiratory inhibitors were significant on all traits in the one percentage probability level. The maximum amount of the traits of the fruit quality scores on the sixth and last days of testing and the total dissolved solids all were observed in control fruit, and the fruit weight loss in CN alone on the final day of testing. The lowest rate of the weight loss of the fruit on the final day of testing and two traits of the fruit quality scores were related to both salicylhydroxamic acid and potassium cyanide together, but the total dissolved solids (brix) were observed just in SHAM alone. These findings suggest that alternative pathway in post-harvest banana fruit ripening, or the Climacteric Respiration, shows dramatic, interesting and significant effect.

Key words: banana, ripening, alternative oxidase, and brix

Received 20.03.2014

Revised 02.04.2014

Accepted 09.06. 2014

INTRODUCTION

Cyanidric acid and cyanides are a bunch of nitriles that from chemical point of view, belong to the n-hydrocarbon compounds with the formula R-CN. The CN factor is highly toxic and can cause tissue asphyxiation. The cyanide products are used in photography, laboratory reactions, industry, insecticides and rodenticide (HCN). Some fruits such as almond, apple seed, and peach and plum seed contain cyanide [1]. Fruit ripening is a complex, genetically programmed process that culminates in dramatic change in colour, texture, flavor, and aroma [2,3]. Fruit with different ripening mechanisms can be divided into two groups: climacteric fruit, in which ripening is accompanied by a peak in respiration and a concomitant burst of ethylene, and non-climacteric fruit, in which respiration shows no dramatic change and ethylene production remains at a very low level. The oxidase provides an alternative route for electrons passing through the electron transport chain to reduce oxygen. However, as several proton-pumping steps are bypassed in this alternative pathway, activation of the oxidase reduces ATP generation. This enzyme was first identified as a distinct oxidase pathway from cytochrome c oxidase as the alternative oxidase that is resistant to inhibition by cyanide [4]. Many experiments were performed regarding the respiratory alternative pathway in different applications and kinds; among them was an experiment which was performed as *Lepidium sativum* seed priming by respiratory inhibitor materials that brought about the maximum rate of traits of dry weight and stem height related to salicylhydroxamic acid and CN. However, the minimum rate was observed in control plants [5]. Another experiment, Abadikhah [6], was conducted in which respiratory inhibitor materials were used instead of water to irrigate the plant. The highest dry weight of *Lepidium sativum* was related to salicylhydroxamic acid and CN, with an average of 0/017 grams. The lowest dry weight observed in control plants with an average of 0.007 grams which was not significant with salicylhydroxamic acid alone. Then, the electron transport through the alternative pathway increased under phosphate limitation [7], during the chilling recovery [8]. Abadikhah et al. [9] reported that the highest pH at 4°C temperature was in control plants, and the lowest pH was obtained

bysalicylhydroxamic acid and CN. Salicylhydroxamic acid was the highest and salicylhydroxamic acid and CN were the lowest at the 0°C on Wallflower. In another experiment conducted on isolated mitochondria, activation of the AOX by pyruvate or by an increase on the reduction status of the disulfide bridge caused an increase in AOX activity but an increase in total oxygen uptake [10, 11]. The highest pH at -4 °C temperature in control, and the lowest was obtained by salicylhydroxamic acid and CN [9]. Some studies have indicated the potential of alternative pathway in thermogenic plants. For example in the experiment conducted by Raskin *et al*, [12] salicylic acid was identified as the natural trigger of heat production in thermo genic plants. A transient, nearly 100-fold increase in the levels of Salicylic acid in thermogenic tissues results in higher alternative pathway activity and temperature increase up to 12°C [12, 13]. Lambers, [14] believes that alternative pathway is an energy overflow and oxidizes the respiration substrates surplus to growth requirement. Moreover, several experiments were conducted on fruit ripening. In one of them the total respiration (Vt) changed little from the mature green (MG) to the turning (T) stage. It was maximized at the pink (P) stage and subsequently decreased. CN-insensitive respiration (Valt) followed a pattern similar to that of Vt. Valt increased 3-fold from the MG to the P stage when it accounted for 40% of the Vt at the P stage. The results suggest that the AOX pathway respiration may contribute to the respiration climacteric during fruit ripening [15].

In addition, Xu *et al*, [15] observed retarded ripening; reduced carotenoids, respiration, and ethylene production; and the down-regulation of ripening-associated genes with reduced *LeAOX* levels in tomato plant. The respiration of a fresh pre-climacteric banana slice is six to eight times that of the intact organ [16] and quantitatively similar to that of the climacteric fruit. Slicing an intact banana in mid-climacteric results in a 38% increase of respiration, bringing the respiration to that of the peak climacteric fruit and pre-climacteric slice [17]. The transgenic plants with reduced *LeAOX* levels failed to ripen after 1-methylcyclopropene 1 treatment, while such inhibition was notably less effective in 35S-*AOX1a* fruit [15]. The present study however was performed to understand the influence of this route on the process of ripening of cut banana fruit.

MATERIALS AND METHODS

The present study was conducted in a One-Way ANOVA design on cut fruits banana with four smeared treatments (the skin fruits were smeared by these treatments with soaked cotton): 1) without respiratory inhibitors as control, 2) potassium cyanide, 3) salicylhydroxamic acid with concentration of 0.005 mM and 4) KCN and SHAM in 3 replications. After preparation of the above solutions, they were normalized by HCL and NH₄OH, like distilled water [18]. In addition, distilled water was used as control solution. To ensure and remove the possible differences between the fruits, the fruits were divided into three similar parts. The measured traits included: fruit weight loss at the last day, total dissolved solids (brix), and the fruit quality scores on the sixth and last days. To measure the trait of weight loss, all the fruits were weighted at the beginning and at the end of the experiment. The first weight was subtracted from the last one.

Each day from the first day to the last one, the apparent change of colour and quality of fruit ripening were scored. Finally, the traits of the fruit quality on sixth and last days were reported by scores assigned to them.

After normalizing and analyzing the data by Minitab software, the averages were compared through Duncan's multiple range test at 5% level.

RESULT

Fruit weight loss on the last day

Analysis of variance for traits of fruit weight loss on the last day (Table 1) showed that the effect of respiratory inhibitors was significant at one percentage probability level. So, the respiratory inhibitor on the trait of fruit weight loss affected differently, and the Comparison of average (Table 2) indicated that the highest fruit weight on the last day was related to potassium cyanide with an average of 60.94 gram that was not significant with the other treatments at the five percent probability level. The lowest fruit weight loss rate was related to salicylhydroxamic acid with an average of 50.02 g that with the other treatments was significant at the five percent probability level. All these demonstrates that two reasons for this result can be probably notable :the first is that the transpiration or fruit dry weight was low and both leading to fruit fresh weight increase.

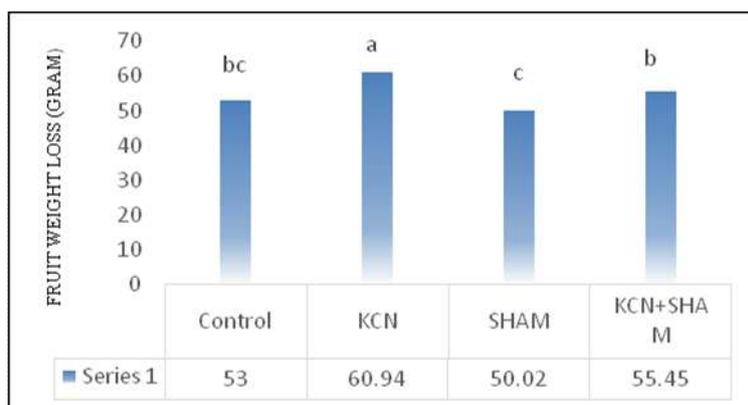


Figure1- Mean of fruit weight loss under different respiratory inhibitors

As the treated fruits with respiratory inhibitors and having contact with the skin and pulp of the fruits because influx of material into banana, and banana belongs to climacteric plants, these plants need material change to complete their ripening period. As CN causes oxidase pathway (AOX) blockage and plants to supply their energy use alternate pathway (cyanide-resistant), which have lower energy at shorter time to continue the respiration? Consequently, the treated fruit uses more substrate than the control fruit to supply their energy, so it is expected that the treated fruit by potassium cyanide may lose more weight than by other treatments. It was notified that SHAM had lowest fruit weight loss rate. As reported in the experiment conducted by Xu *et al.*, [15]. AOX reduction in tomato fruit increased the fresh weight, likewise, the result of the present study indicated that the lowest fruit weight loss rate was by salicylhydroxamic acid. Removal of AOX pathway can cause loss of fruit water [15], as a result, with reduction of the AOX pathway by SHAM, the increase of the fresh weight was noticeable. In the present experiment, reduction of the AOX could cause water loss, therefore the weight gain obtained was not due to the inside water, then increase of dry weight is probably because of the fruit fresh weight increase. It should be added that there are also other reasons that will be explained in other traits.

Table1-Analysis of variance mean squares of the traits in **fruit** banana

	D.F	fruit quality score in last day	fruit quality score in day 6	TSS	Weight loss on last day
Replication	2	3.00	3.25	0.02	5.03
Treatment	3	45.00**	45.00**	0.88**	64.16**
Error	6	0.33	0.25	0.004	5.13

*, **, and Ns indicate 5%, 1% significance level and not significant respectively.

Total dissolved solids (brix) on the last day

Trait of TSS on the last day (Table 1) showed that the effect of respiratory inhibitors was significant at 1 % probability level ($p < 0.1$). The application of respiratory inhibitors affected the TSS differently, and the mean comparison (Table 2) indicates that maximum total dissolved solids rate achieved from control fruit with an average of 8.55% that was significant with the other treatments at the five percent probability level. The lowest amount of total dissolved solids was related to salicylhydroxamic acid with an average of 7.31 % that was significant with the other treatments at the five percent probability level.

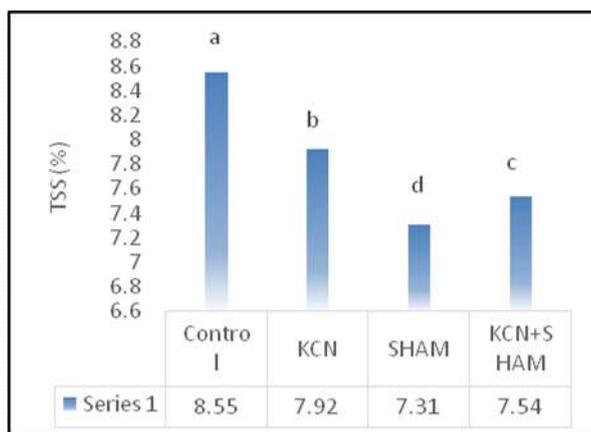


Figure2- Mean of TSS under different respiratory inhibitors

As previously indicated, when the fruits are ripening, especially in climacteric-type fruits, the more the fruit going through the ripening process the more glucose is obtained. The control fruit was a treatment that went through the ripening process earlier than other treatments. So, sugar, in other word, TSS showed the maximum TSS rate, and the minimum TSS rate was related to SHAM that matched with the results of other researchers in this field. For example Xu *et al.*, [15] observed that remove of the AOX pathway lead to longevity of ripening process after harvesting, in which SHAM had more time than the control fruit, then TSS was at its lowest level.

Table2- Comparison of mean of fruit banana traits

treatment	fruit quality score in last day	fruit quality score in day 6	TSS (%)	Weight loss on last day (g)
Control	11 a	11 a	8.55 a	53.00 bc
KCN	8 b	8 b	7.92 b	60.94 a
SHAM	5 c	5 c	7.31 d	50.02 c
KCN+SHAM	2 d	2 d	7.54 c	55.45 b

Similar letters in every column mean that they are not significant at 5 % probability level.

Fruit quality scores on the sixth day

Results showed that trait of fruit quality scores on the sixth day was significant at 1 % probability level ($P < 0.1$). The highest amount of this trait achieved from control fruit. From Statistical point of view a significant difference was observed compare to other treatments, in other words, the highest score indicated the fastest ripening process.

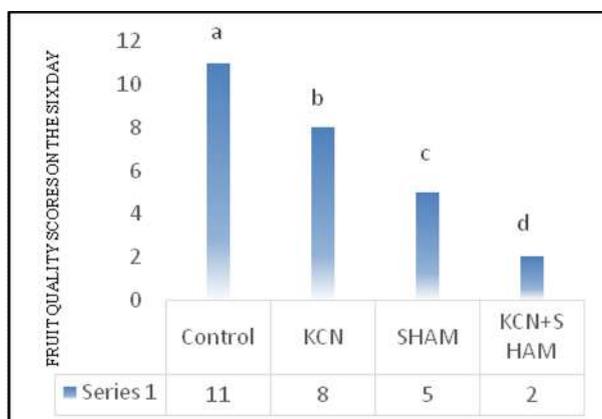


Figure3- Mean of fruit quality scores on the sixth day under different respiratory inhibitors

From another perspective, it can be said that the respiratory inhibitors cause longevity of ripening process in the postharvest fruit compare to other treatments, which is similar to other experiments. As

described already, Xu *et al.*, [15] observed that remove of the AOX pathway lead to longevity of ripening process after harvesting, the same result was achieved at the present experiment in which, SHAM reduced alternative path that caused retard of the ripening process. CN alone had more deterrence effect, which can be related to the production of ethylene, but further investigation is needed.

Fruit quality scores on the last day

Analysis of variance revealed that the fruit quality scores on the last day was significant at the 1 % probability level ($P < 0.1$). The measuring goal of this trait, was investigation of how to change in terms of ripening process during the ripening period, which finally indicated that these two pathways were the same.

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Citation of This Article

Danial A deh-ali, Ahmad M, Maryam M, Neda H. Physiological effects of CN-resistance Alternative Pathway on Climacteric Respiration of Banana fruit. *Bull. Env. Pharmacol. Life Sci.*, Vol 3 [8] July 2014: 84-88