Plant Science 252 (2016) 193–204

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Short versus long term effects of cyanide on sugar metabolism and transport in dormant walnut kernels

Zahra Gerivani[a], Elham Vashaee[a], Hamid Reza Sadeghipour[a,*], Mahnaz Aghdasi[a], Zahra-Sadat Shobbar[b], Majid Azimmohseni[c]

[a] Department of Biology, Faculty of Science, Golestan University, Gorgan, Iran
[b] Molecular Physiology Department, Agricultural Biotechnology Research Institute of Iran, (ABRII), AREEO, 3135933151 Karaj, Iran
[c] Department of Statistics, Faculty of Science, Gorgan, Iran

ARTICLE INFO

Article history:
Received 24 April 2016
Received in revised form 25 July 2016
Accepted 27 July 2016
Available online 30 July 2016

Keywords:
Cyanide
Gluconeogenesis
H-ATPase
Juglans regia
Seed dormancy
Sugar transport

ABSTRACT

Tree seed dormancy release by cold stratification accompanies with the embryo increased gluconeogenesis competence. Cyanide also breaks seed dormancy however, integrated information about its effects on carbon metabolism is lacking. Accordingly, the impacts of HCN on germination, lipid gluconeogenesis and sugar transport capacity of walnut (*Juglans regia* L.) kernels were investigated during 10-days period prior to radicle protrusion. HCN increased walnut kernel germination and within four days of kernel incubation, hastened the decline of starch, reducing and non-reducing sugars and led to greater activities of alkaline invertase and glucose-6-phosphate dehydrogenase. From four days of kernel incubation onwards, starch and non-reducing sugars accumulated only in the HCN treated axes. Cyanide also increased the activities of phospho*enol*pyruvate carboxykinase and glyoxysomal succinate oxidase and led to greater acid invertase activity during the aforementioned period. The expressions of both sucrose transporter (*JrSUT1*) and H^+-ATPase (*JrAHA1*) genes especially in cotyledons and H^+-ATPase activity in kernels were significantly enhanced by exposure to cyanide. Thus in short-term HCN led to prevalence of carbohydrate catabolic events such as oxidative pentose phosphate pathway and possibly glycolysis in dormant walnut kernels. Long-term effects however, are increased gluconeogenesis and enhanced sugar transport capacity of kernels as a prerequisite for germination.

1. Introduction

Seeds from many plant species are unable to germinate under suitable moisture and temperature conditions, a phenomenon known as dormancy. Dormancy endows plant species survival against natural adverse environmental conditions. The inability of seeds to germinate is either due to embryo morphological and/or physiological immaturity or to other ex-embryo seed constraints such as seed coat and endosperm [1]. The release of seed dormancy which finally leads to germination is affected by both exogenous factors such as temperature and moisture and endogenous factors including phytohormones [1]. Furthermore other reactive molecules such as NO, H_2O_2 and HCN with either endogenous or exogenous origins can affect this process [2,3].

There are some progresses in understanding the molecular mechanisms of cyanide action in releasing seed dormancy. Exposure of dormant sunflower seeds to gaseous cyanide for a few hours leads to the alleviation of embryo dormancy and germination [4]. This dormancy-breaking effect of cyanide is due to inhibition of reactive oxygen species (ROS) scavenging enzymes like catalase and superoxide dismutase as well as the stimulation of NADPH-oxidase activity which is responsible for the generation of superoxide anions [5]. The resulting upsurge of various ROS forms especially hydrogen peroxide is proposed as an essential signal for breaking seed dormancy [6]. By targeted oxidation of mRNAs and proteins associated with their altered functioning, various ROS forms are implicated to provoke dormancy releasing events in seeds and bring about germination [7–10]. In congruence, the hydrogen peroxide-mediated dormancy release of some investigated seeds is achieved through the up-regulation of ABA catabolism, and GA biosynthesis genes [11–13]. Cyanide effect on seed dormancy removal in addition, shares some response components of ethylene

Abbreviations: GSO, glyoxysomal succinate oxidase; OPPP, oxidative pentose phosphate pathway; PEPCK, phospho*enol*pyruvatecarboxykinase.

* Corresponding author.
E-mail addresses: zahragerivani@gmail.com (Z. Gerivani), elham.vasha@gmail.com (E. Vashaee), h.r.sadeghipour@gmail.com (H.R. Sadeghipour), aghdasi46@yahoo.com (M. Aghdasi), shobbarz@yahoo.com (Z.-S. Shobbar), azim_mohseni@yahoo.com (M. Azimmohseni).

http://dx.doi.org/10.1016/j.plantsci.2016.07.016
0168-9452/

signaling [5]. The emerging picture for cyanide action in the promotion of seed germination of woody species is with some variations similar to those reported for herbaceous ones. Thus, in apple (*Malus domestica* Borkh.) embryos, cyanide application leads to ROS accumulation, stimulation of ethylene biosynthesis [14] and enhanced degradation of carbonylated proteins [10].

Following dormancy release, the growth of organs like radicles in germinating seeds is mainly associated with cell elongation processes, which demands the action of a plasma membrane H^+-ATPase for proton transport into apoplast and cell wall acidification [15–17]. Furthermore, the functioning of H^+-ATPases leads to a trans-membrane pH difference which acts as driving force for processes such as secondary active transport [18,19]. The activity of various H^+-ATPases seems to be important during seed germination and seedling growth. Thus seedlings of a high-vigor maize line display greater tonoplast H^+-ATPase activity compared to a low-vigor line [20]. Furthermore, the activity of a plasma membrane H^+-ATPase decreases in roots of maize seedlings derived from aged seeds [21]. The plasma membrane H^+-ATPases in association with sucrose transporters and sucrose metabolizing enzymes are also the major players regulating source-sink relationships in the whole plant [22]. Positive correlations between H^+-ATPase activity, maize plasma membrane sucrose transporter (*ZmSUT1*) expression, apoplast acidification and sucrose mobilization in imbibing maize embryos support the cooperation of H^+-ATPase and SUT1 in sugar transport from the scutellar source tissues to the growing radicle [23]. Sucrose transporters OsSUT1 and TaSUT1 are expressed in germinating rice and wheat seeds, respectively [24,25]. There are also other roles for sucrose transporters in germination related processes. In *Populus sp*., the tonoplast localized SUT4 is an important component regulating the plant water relations [26]; a feature which would be important during seed imbibition and germination processes, since the action of sucrose transporters provide sugars for cell wall biosynthesis and production of osmotic pressure [27].

Based on 'metabolic inhibition theory' seeds with physiological dormancy have some metabolic failure in utilization of their own food reserves which can be overcome after exposure to dormancy releasing treatments such as cold stratification or dormancy breaking chemicals [28,29]. Thus the alleviation of seed dormancy by cold stratification accompanies with the activation of lipid mobilization and gluconeogenesis [30,31], priming of proteolytic processes [31,32], and the enhanced mobilization of other seed reserves such as phytate [33]. There is no integrated information about the impact of cyanide application on the carbon metabolism of dormant seeds. The exposure of dormant sunflower seeds to cyanide was not associated with significant changes in the level of sucrose and monosaccharides like glucose and fructose; thus its role in gluconeogenesis activation was ruled out [5]. In dormant apple embryonic axes cyanide application stimulates early mobilization of sucrose and raffinose through the enhanced activity of alkaline invertase [34], and priming of glycolysis as evidenced by the increased activity of pyruvate kinase [35], PPi-dependent phosphofructo kinase and fructose 6-phosphate 2-kinase [36]. Proteomic studies have also shown the build-up of several proteins related to oxidative pentose phosphate pathway (OPPP), reserve utilization and gluconeogenesis in seeds treated with different dormancy releasing agents [37–40].

Walnut (*Juglans regia* L.) is an economically important tree species from temperate regions which is cultivated for its nutritive nuts, though its timber is also industrially important. The commercially available nuts consist of a sclerified shell enclosing a bulky kernel i.e. an embryo enriched of lipids (up to 70%), proteins and carbohydrates [41]. Kernels have physiological dormancy which can be alleviated by cold stratification [42]. In contrast to other studied tree seeds [29,32,33], storage oil and protein mobilization can proceed in dormant walnut kernels just upon imbibition while the gluconeogenesis of lipids and amino acid metabolism remain inefficient [42–45]. Under stratified cold conditions however, the kernel gluconeogenesis is activated through down regulation of mitochondrial dehydrogenases and the stimulation of both glyoxysomal succinate oxidase (GSO) and phospho*enol*pyruvate carboxykinase (PEPCK) activities [46]. Accordingly, it was of interest to see whether the dormancy release of walnut kernels by cyanide if any, is also associated with the increased kernel competence for gluconeogenesis of lipids and transport of the resulting sugars from cotyledons to axes. The obtained results allowed us to propose a model of cyanide action on carbon metabolism of walnut kernels during the alleviation of dormancy. All measurements were carried out during a critical stage of seed germination i.e. before radicle protrusion to realize the metabolic preparations undergoing in kernels for exit from dormancy.

2. Materials and methods

2.1. Plant material and germination studies

Freshly harvested seeds of Persian walnut (*Juglans regia* L.) were procured during October of 2012, 2013 and 2014. Kernels not older than eight months after harvest were used for studies. Seventy five nuts in triplicates of 25 were pierced at the peduncle pole of their stony shell, soaked in tap water for 24 h, and treated with 1 mM gaseous hydrogen cyanide (HCN) as described by Oracz et al. [4] with some modifications. For this, nuts were transferred to a 3.6 l Pyrex glass jar lined with moist filter paper within which was a beaker containing 0.1 M KCN solution (36.0 ml). The collection was then tightly covered with a polypropylene cape and sealed. Gaseous HCN evolution within the jar was achieved after discharging 36.0 ml lactic acid (10%, v/v) into the beaker containing KCN solution. For this the cape was pierced with a hypodermic syringe just above the beaker and the residual pore due to needle scar was sealed. The jar which already contained evolved HCN was left in darkness. The calculated HCN concentration at equilibrium was then 1.0 mM supposing that all cyanide from the KCN has been evaporated and distributed uniformly. After 4 h nuts were taken out, washed with plenty of tap water to remove the residual cyanide and used for subsequent experiments. Nuts soaked in tap water (28 h) but not exposed to hydrogen cyanide were used as control. Both hydrogen cyanide treated and control nuts were surface sterilized with 0.5% (w/v) sodium hypochlorite solution for 15 min followed by four times washing with distilled water. They were then transferred into a moist sand medium at 27° C for up to 35 days and recorded for germination. Kernels having a radicle length of about 1.0 cm were regarded as germinated.

Cold stratification of walnut kernels was carried out as described earlier [42]. Cold stratified kernels (for 30 days) with no sign of germination were directly used for biochemical analyses, indeed for assessing their germination potential they were transferred to sand medium as described above for HCN exposed kernels.

All biochemical and molecular studies were carried out on at least three tissue samples from both distal cotyledonary lobes and apparent axes of kernels not showing any sign of germination during a 10 days period (started from nut transfer into sand medium i.e. before radicle emergence). The reported values for the results of both gene expression and biochemical studies are the mean of three separate kernel extraction and assay ± SE.

2.2. Determination of lipid and carbohydrates

Extraction and quantification of total lipids from walnut kernels were carried out according to the method of Hara and Radin [47] as described earlier [42]. The defatted powder obtained following