Review

Plant nuclear proteomics for unraveling physiological function

Xiaojian Yin[1,2] and Setsuko Komatsu[1,2]

[1] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan
[2] National Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba 305-8518, Japan

The nucleus is the subcellular organelle that functions as the regulatory hub of the cell and is responsible for regulating several critical cellular functions, including cell proliferation, gene expression, and cell survival. Nuclear proteomics is a useful approach for investigating the mechanisms underlying plant responses to abiotic stresses, including protein–protein interactions, enzyme activities, and post-translational modifications. Among abiotic stresses, flooding is a major limiting factor for plant growth and yields, particularly for soybean. In this review, plant nuclei purification methods, modifications of plant nuclear proteins, and recent contributions to the field of plant nuclear proteomics are summarized. In addition, to reveal the upstream regulating mechanisms controlling soybean responses to flooding stress, the functions of flooding-responsive nuclear proteins are reviewed based on the results of nuclear proteomic analysis of soybean in the early stages of flooding stress.

Contents

Corresponding author: Komatsu, S. (skomatsu@affrc.go.jp)

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Introduction

Because of the importance of the nucleus [1–4], the proteomic analysis of nuclear proteins has attracted considerable recent interest and the findings from plant nuclear proteomic studies have been reviewed [5–7]. To date, the nuclear proteome of many plant species, including *Arabidopsis* [8], *Medicago* [9], rice [10], chickpea [11], tomato [12], potato [13], apple [13], maize [14], *Xerophyta viscosa* [15], hot pepper [16], barley [17], wheat [18,19], and soybean [20], has been analyzed. Soybean, which is cultivated worldwide, provides an abundant source of protein and edible vegetable oil for human consumption [21,22]. Although soybean is able to grow in a wide range of climatic conditions, plant growth and grain yield are greatly reduced by flooding [23], which is typically caused by heavy or continuous rainfall in areas with poorly drained soil [24]. In response to flooding stress, soybean displays altered patterns of gene expression, protein abundance, and metabolite accumulation [25]. To identify the upstream events controlling the regulation of flooding-responsive proteins in soybean, the proteomic analysis of nuclear proteins is a powerful technique.

Proteomic techniques allow for the systematic analysis of complex cellular mechanisms, such as plant responses to abiotic stress [25], as proteomic analyses provide qualitative and quantitative data on protein properties, including intracellular distribution, turnover dynamics, protein–protein interactions, and post-translational modifications [26]. Determination of the nuclear proteome provides insight into the complex regulatory networks that function in plant nuclei. Recent advances in nuclear proteomic techniques have allowed for the ever-more sensitive detection of nuclear proteins. Using these approaches, nuclear proteins that are modulated in response to flooding stress were extensively characterized in soybean [27–30].

In the present review, methods for nuclei purification from various plant species are described and common modifications of plant nuclear proteins are presented. Furthermore, to provide insight into the upstream mechanisms regulating the early soybean responses to flooding stress, the functions of flooding-responsive nuclear proteins are discussed.

Purification of nuclei

Composition of homogenization buffers used for nuclei isolation

For the purification of plant nuclei, homogenization buffers with variable compositions depending on the type of plant material and intended downstream analysis have been used (Table 1). The basic requirements for a suitable homogenization buffer are that the integrity of the nuclear membrane and stability of nuclei must be maintained during the isolation of released nuclei from intact cells. To stabilize the pH of homogenization solution, organic buffers such as MES, HEPES, PIPES, and Tris are typically used. Inorganic salts, such as KCl and NaCl, are used to maintain the ionic strength of the solution, and sucrose, glycerol, and hexylene glycol are added to stabilize membranes [31]. In addition, polyamines are used to stabilize the nuclear chromatin and avoid the aggregation of nuclei [32]. Reducing agents, such as 2-mercaptoethanol and dithiothreitol, which maintain cysteine residues in the reduced form, are also important components of homogenization buffers. The addition of protease inhibitors to nuclei isolation buffer is also important for protecting proteins from degradation during the purification process [33]. Furthermore, resins capable of absorbing phenolics have been used for the isolation of nuclei from plant species that produce large amounts of phenolic compounds [34].

For the study of plant nuclear components and events, the isolation of nuclei is a critical step, and specific methods are needed to purify nuclei from the plant organs of different species (Table 1). For example, homogenization buffer composed of hexylene glycol and PIPES-KOH in combination with discontinuous percoll gradients is typically used for the isolation and purification of nuclei from *Arabidopsis* cotyledon/leaf and chickpea seedlings [8,33,35], whereas homogenization buffer composed of HEPES-KOH and continuous sucrose gradients was used to purify nuclei from rice seedlings and soybean root tips [28,36,37]. For nuclei isolation from potato and apple leaves, homogenization buffer composed of MES-KOH and combined continuous percoll and sucrose gradients was successfully applied [13]. In the case of tomato fruit and maize leaf, homogenization buffers composed of Tris-HC1 in combination with continuous sucrose gradients were used [12,14].

Basic procedure for nuclei isolation

The separation and enrichment of nuclei from plants is technically challenging, and contamination of the nuclear fraction with proteins from other subcellular organelles impedes the analysis of nuclear proteins [34]. Several different techniques for isolating plant nuclei have been developed. Most of the isolation methods consist of similar sequential steps that include disruption, filtration, centrifugation, solubilization and separation (all performed on ice). The final two steps, which involve removal of membranes from contaminating organelles using non-ionic detergents and the separation of nuclei by density gradient centrifugation, are critical for obtaining highly purified nuclei. The use of non-ionic detergents, such as Triton X-100, promotes the release of nuclei from cells and prevents nuclei from forming aggregates [38]. Moreover, detergent application aids in the isolation of nuclei from green leaves, as leaves contain abundant chloroplasts, which are sensitive to breakdown and solubilization by detergent [13]. However, the plant nuclear membrane is easily damaged by high concentrations or prolonged exposure to detergent [39]. The density gradient systems used for isolation of nuclei vary depending on the plant species; however, the density medium of these systems typically consists of sucrose, percoll, or mixtures of sucrose and percoll.