DISTRIBUTION OF POLYAMINE ON SOYBEAN GROWN UNDER ACIDIC STRESS

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INTISARI

Distibusi poliamina pada daun kedelai dari bebagai tingkat perkembangan fisiologis yang mana kedelainya ditanam pada kondisi stres asam (pH tanah 5.1) telah dianalisa dengan HPLC fasa balik dengan UV/Vis spektrofotometer sebagai detektor. Data memperlihatkan adanya perbedaan distribusi poliamina pada berbagai tingkat perkembangan fisiologis tanaman. Keadaan cekaman asam juga menyebabkan perbedaan distribusi dan ekspresi poliamina pada kedelai. Sembilan senyawa amina biasa (common) yaitu putrescina dan spermidina, dan tujuh senyawa amina lainnya terdeteksi didalam daun kedelai yang ditanam pada tanah ber pH netral (pH 6,7). Disamping polyamina biasa, dua polyamina tidak biasa (uncommon) yaitu caldina dan satu poliamina tidak dikenal lainnya ditemukan pada kedelai yang ditanam pada tanah masam (pH 5,1). Diduga kedua poliamina tidak biasa ini diinduksi oleh asam sebagai mekanisme tumbuhan dalam mengatasi stres asam.

ABSTRACT

The distributions of polyamine on the leaf of soybean grown under acidic stress at different stage of plant development have been analyzed by reveres phase HPLC with UV/Vis spectrophotometer as detector. It was shown that the distribution of polyamine at different stage of plant development seems to be different. Nine amines compound namely putrescine and spermidine, and seven other unknown amine compounds were detected on the leaf of soybean grown under neutral pH. Two different polyamines were found to be induced by acidic stress that are caldine- an uncommon polyamine, and an unidentified amine compound. It is postulated that these two uncommon polyamines are induced as a plant mechanism to overcome the acidic stress.

INTRODUCTION

Several aliphatic di- and polyamines such us putrescine, spermidine, and spermine are common and ubiquitously distributed in living cells in titer raging from approximately 10 μM to milimolars[1]. These polyamines are designated as common polyamines. Putrescine and spermidine are found in all prokaryotic and eukaryotic cells, while the tetramine, spermine, with few exception is only detected in eukaryotic cells[2]. Many other aliphatic di- and polyamines have limited distribution in nature, and thus are termed uncommon or unusual polyamine. Sym-norspermine (1, 11-diamino-4,8-diazaundecane), and norspermine were identified as component of polyamine pool in *Caldariella acidophila*, an extreme thermoacidophillic bacteria[3].

There are many possible role of polyamine in cell have been reported. Certain common polyamines were found to be essential for both prokaryotic and eukaryotic organism. Spermidine and spermine are involved in the regulation of cell growth and proliferation[4]. A normal polyamine complement is essential for the progression through the various phase of the cell cycle[5]. Simultaneously inhibition of spermidine and spermine biosynthesis prevented the germination process of *Picea abies* seeds[6].

Polyamine have been considered as a new class of plant growth regulator, or second messenger, since they are translocatable, a phytohormone criterion, in the whole plant[7]. Polyamines like phytohormone such as ABA, regulate long distance transport of potassium in plantlets of *Solanom tuberosum*[8].

Several evidence have shown the role of polyamine in adaptation mechanism of the cells to a biotic stress. The change of polyamine titer have been reported in response to wide variety of abiotic stress in many different plants. Several uncommon polyamines such as caldine and thermin have been detected in organism grown under abiotic stress such as heat and drought. Norspermine was detected as a minor polyamine component in *C. acidophila*,[3] and in cotton and

alfalfa meristem tissue subjected to drought and /or heat stress[9]. Polyamine interact and stabilize the nucleic acid and ribosome.

Large portion of Indonesian soil is acidic. How the stress by acidic soil affect the distribution of polyamine in soybean is one of he question to solve in our research. The long-term target of the research is to identify, isolate and study the gene for the key enzyme responsible in biosynthesis of the polyamine, induced by acidic stress.

EXPERIMENT

Preparation of Sample

The soyabean (Kedelai Kipas putih) were grown on acidic soil (pH 5,1) and on neutral soil (pH 6,7). The leaf of Soya plants were harvested at different physiologic stage, e.g. germination stage, flowering stage and fruiting stage. The harvested leafs were kept immediately on ice temperature (4 °C). The leafs were stored on refrigerator for longer period of storage.

Polyamine Isolation

Frizzed leafs of soy plant were homogenized in trichloroacetic acid (TCA 5%) (1 g leafs : 2 ml TCA 5%) on the blender. The homogenate was centrifuged at 12,5000 g for 20 min, and the supernatant was decanted and stored as an crude extract of polyamine. Next, the polyamines were isolated by cation exchange resin. The polyamine crude extract (1ml) were homogenized with active-cation exchange resin (3 g) by agitation for 1 hr at 100 rpm. The resin were filtered on Whatman filter paper. Next he polyamine were eluded from the resin by agitation of the mixture of resin with HCl 11 M for 2 hr at 100 rpm. The eluded polyamine were filtered and kept in the tube. Tube containing the eluted polyamine were placed in warm (40-60 C) bath in fume hood in order to dry the sample and evaporate the acid. To accelerate the evaporation a gentle stream of air was directed over the top of each tube. It took about 24 hour to co-plate the drying process. Each tube contained a solid residue after drying which include the hydrochloride salt of isolated polyamine.

The Soluble crystal were test qualitatively for polyamine. The solution was spotted on the filter paper and was let them dry. After drying, the spot were sprayed with ninhidrin 10%. The polyamine spot will turn blue with ninhydrin.

Analysiis of Polyamine with High Performance Liquid Chromatography (HPLC)

Polyamine cristal were dissolved quantitatively with deionized distilled water, and then each of 400 ml solution was added with 100 ml of 5 N Na_2CO_3 and vortexed. Next, 500 ml dansyl chloride solution (200 mg dansyl chloride in 30 ml acetone) was added to the polyamine solution and vortexed. The solution was incubated on water bath at 54 °C for 1 hr to accelerate the dansylation of polyamine and evaporate the acetone. The excess of dansyl chloride was reacted by addition of 100 @L of 1.0 M L-proline followed by incubation in a 25 °C water bath for 30 min. Dansylated polyamine were extracted into 1 ml of dry benzene (distilled and stored over anhydrous calciun sulfate). The top benzene layer of extraction mixture was collected for each sample over vigorous vortexing. This step was repeated 4-5 times with 1-ml portion of dry benzene to enhance the extraction yield. To remove benzene from the sample, extract were pooled and evaporate to dryness in hood with air stream. The dansylated polyamine was dissolved in 500 @L of 80% ethanol /20% water. All prepared dansylated polyamine solution were filtered into epitubes.

HPLC analysis was conducted with a system consisting of a Perkin Elmer pump LC 250 and detector ultraviolet, visible spectrophotometer detector model LC 95. The separation of polyamine was conducted with isocratic condition. HPLC mobile phase solvent were deionized, distilled water and HPLC grade methanol.

Sample (30 uL) was injected and eluded with methanol 80% with the flow rate 0,5 ml/ min for 1 hr through column partisil ODS-3. Dansylated polyamine were detected by absorbance detector at λ 254 nm.

RESULT AND DISCUSSION

Polyamine Analysis On Soybean Grown At Neutral pH

There were nine peaks of amine compound were detected in the sample of soybean grown under neutral pH (Table). However only two amine compound that could be identified based on of their retention time, they were putrescine (Put) and spermidin (Spd). The other peak of amine compounds could not be detected because of lack of the polyamine standard. Unidentified amine compound were designated with amine compound A, B, C, D, E, F,and G.

Table : Distribution Of Polyamine In Soybean In Acidic And Neutral Soil At Different Stage Of Development which Shown as a Peak area of Chromatogram

Acid/Age*	Put	A	B	C	D	Cal	Spd	F	F
N-4	5082	3263	3475361		890				
N-8			3497237		4527				3058
N-27	85050	21445	4326929		21765		2161	1079	23568
N-31	13895	13514	1873653		7674				12263
N-33	24928	13263	1083900		61435		3451		45974
N-39			2393389		3594				
S-4		25274	2513079	587				70990	
S-8	331		1051397		1244				2041
S-27	60828		8284936		14406				21288
S-31	13606	16097	52935				12869		
S-36	16590		1479642		2036	4317			
S-39	17394		983843567		2209				
S-43	34717	10504	4995		31474				31383

* Acid mean acidity of soil on where the soybean was grown and Age mean age of plant on days. N and S mean plant was grown on neutral (pH 6.7) soil, and under stress (low pH soil, pH 5.1) respectively. e.g. A-4 = Plant grown in Acidic soil for 4 days, N4= Plant grown in neutral soil for 4 days. A-F = Unidentified amine compound

The abundance of these amine compound were different at different stage of physiologis condition. Putrescine peak was found at the stage of growing or sprouting. The biosynthesis of putrescine seems to shift to the biosynthesis of spermidin after 8 days. The unidentified amine compound B was detected at all of stage of physiologis conditionm of plant. Putrescine and amine compound D were also detected in almost all of the sample, both on plant grown on acidic and neutral soil. Spermidine was detected on both plant, grown under acidic and neutral soil, however the acidic soil seems to induce the biosynthesis of spermidine. There are two amine compound that were detected on plant grown under stress of acidic soil, but were not detected on the plant grown on neutral soil, they are caldine and amine C. These amine compound might function on the mechanism of plant to overcome of the acidic stress.

CONCLUSION

1. The distribution of polyamines are different at different stage of development of soybean
2. Acidic stress seems to induce the biosynthesis of uncommon polyamine caldine and unidentified amine compound C.
3. Caldine might play a role on mechanism of plant to overcome the a biotic stress.

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