The natural alkaloid sanguinarine promotes the expression of heat shock protein genes in Arabidopsis

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Title page

SHORT COMMUNICATION

Title

The natural alkaloid sanguinarine promotes the expression of heat shock protein genes in
Arabidopsis

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Abstract

Small-molecule heat shock response inducers are known to enhance heat tolerance in plants. In this paper, we report that a plant alkaloid enhances the heat tolerance of *Arabidopsis*. We investigated 12 commercially available alkaloids to determine whether they enhance the heat tolerance of *Arabidopsis* seedlings by using an *in vitro* assay system with geldanamycin, which is a known heat shock response inducer, as a positive control. Accordingly we found that the isoquinoline alkaloid sanguinarine can enhance heat tolerance in *Arabidopsis*. No such effect was shown for the other 11 alkaloids. The sanguinarine treatment increased the expression of heat shock protein genes such as *HSP17.6C-CI*, *HSP70*, and *HSP90.1*, which were up-regulated by geldanamycin. Treatments with other isoquinoline alkaloids (berberine and papaverine), which showed few heat-tolerance-enhancing effects, did not promote the expression of the heat shock protein genes. These results suggest that sanguinarine influenced the heat tolerance of *Arabidopsis* by enhancing the expression of heat shock protein genes.

Keywords

Alkaloids - *Arabidopsis thaliana* - Geldanamycin - Heat shock protein - Heat tolerance - Sanguinarine

Introduction

The extremely high temperatures caused by heat waves reduce plant production in many areas of the world (Hall 2001; Ainsworth and Ort 2010). One useful approach to suppressing the loss of plant productivity due to heat is to ameliorate the heat stress caused by high ambient temperatures. When plants are exposed to heat stress, various symptoms occur including protein denaturation, the inhibition of protein synthesis, protein degradation, increased fluidity of membrane lipids, the production of reactive oxygen species, and the suppression of photosynthesis (Wahid et al. 2007; Allakhverdiev et al. 2008; Ruelland and Zachowski 2010). In order to reduce the occurrence of such negative symptoms, plants accumulate compatible solutes, alter their hormone and growth regulator contents, enhance their antioxidative activities, and express heat shock proteins (HSPs) (Iba 2002; Kotak et al. 2007). It is believed that the expression of HSP genes is an important factor in establishing heat tolerance in plants (Huang and Xu 2008), because positive correlations between the expression levels of HSP genes and the degree of heat tolerance have been demonstrated (Wang et al. 2004; Wahid et al. 2007; Waters 2013; Zhang et al. 2014). Controlling the HSP expression can be an effective technique to ameliorate the heat damage sustained by plants.

Some low-molecular-weight compounds have shown an ability to enhance heat tolerance in plants. The application of salicylic acid (SA) (Dat et al. 1998; Senaratna et al. 2000), glycinebetaine (GB) (Sakamoto and Murata 2002), polyamines (Wahid et al. 2007), geldanamycin (GDA) (Yamada et al. 2007), and isothiocyanates (ITCs) (Hara et al. 2013) has been shown to enhance the heat tolerance of plants at the laboratory level. Among the known enhancers, GDA has been well characterized in terms of the mechanism by which it enhances heat tolerance. GDA was originally found in *Streptomyces hygroscopicus* as an antibiotic. Since it was demonstrated that GDA shows antitumor activities by inhibiting the HSP known as HSP90s in mammals (Trepel et al. 2010; Roe et al. 1999), GDA came to be used as an HSP90 inhibitor. Active HSP90 negatively regulates the action of heat shock factors (HSFs) by binding them. HSP90 inhibitors like GDA activate HSFs by inactivating HSP90, resulting in the induction of a strong heat shock response. It was proposed that the increase in the heat shock response induced by GDA enhances the heat tolerance of plants (Yamada et al. 2007; Yamada and Nishimura 2008). This suggests that other compounds showing GDA-like activities may enhance the heat tolerance of plants.

GDA is an N-containing macrocyclic polyketide showing antimicrobial activity. This suggests that natural N-containing antimicrobial products such as plant alkaloids may show heat-tolerance-enhancing effects. The alkaloids are one of the most diverse groups of secondary metabolites found mainly in plants (Roberts and Wink 1998). Among them berberine, cocaine, colchicine, mescaline, quinine, and reserpine are examples of antimicrobial alkaloids (Cowan 1999). In order to find alkaloids that show heat-tolerance-enhancing effects, 12 commercially available alkaloids were screened. Accordingly we found that the isoquinoline alkaloid sanguinarine showed a heat-tolerance-enhancing effect.

Materials and methods

Chemicals

Berberine, sanguinarine, reserpine, and trigonelline were purchased from Sigma (Tokyo, Japan). Emetine, noscapine, papaverine, yohimbine, caffeine, quinine, capsaicin, and colchicine were obtained from Wako (Tokyo, Japan). GDA was obtained from LC Laboratories (MA, USA).

Heat-tolerance screening

Solidified culture medium (1/5 MS medium with 0.8% agar and 1% sucrose, pH 5.8) was

prepared in 6-well plates (TPP, Trasadingen, Switzerland, #92406) under sterile conditions. A sterile filter paper disk (No. 1, Advantec, Tokyo, Japan) was placed on the surface of the solid medium in each well. Surface-sterilized seeds of *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia) were sown onto the filter paper at a rate of 10 seeds per well. After vernalization for 2 days at 6°C, the plates were placed in the growth chamber (NK System, Tokyo, Japan) for 6 days under the following conditions: a 16-h day (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$)/8-h night cycle at 22°C. At this step, the seedlings were thinned out to 6 per well. The seedlings with the filter paper were inoculated onto the test medium in the 6-well plates 6 days after germination (DAG). Test compounds were freshly dissolved in dimethyl sulfoxide (DMSO). The concentrations of compounds other than sanguinarine were 0 (control), 0.25, 2.5, 25, and 250 mM. The sanguinarine concentrations were 0 (control), 0.025, 0.25, 2.5, and 25 mM. The test medium (4 ml in each well) was prepared by combining the test DMSO solution (8 μl) and the 1/5 MS medium with 0.8% agar and 1% sucrose (3992 μl). The pH of the test medium was checked using pH indicator strips (Merck, Tokyo, Japan). The plates were incubated under the same conditions as given above for 24 h, and then at 7 DAG the plants were heated as follows. The plates were covered with a polyethylene zipper bag (4-F, 120 nm x 170 mm, System Polymer, Tokyo, Japan) and were totally soaked at 47°C for 1 h. Control plates were placed in the same growth chamber for 1 h. After the plates containing heat-treated and control plants were maintained under the same cultivation conditions for 3 days, the fresh weights of the plants were measured (10 DAG).

Transcripts analysis

Transcripts accumulation of the *HSP17.6C-CI* (*At1g53540*), *HSP70* (*At3g12580*), *HSP90.1* (*At5g52640*), and *ACTIN-2* (*At3g18780*) genes in *Arabidopsis* was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) according to the method given in a previous report (Hara et al. 2013). *Arabidopsis* seedlings were treated with sanguinarine, GDA, berberine, and papaverine at the concentration of 50 μM in the 6-well plates as described above. The 6 DAG seedlings with the filter paper were inoculated onto the medium containing alkaloids, and the seedlings were harvested at 0, 1, 6, and 24 h after the inoculation. To provide the heat shock, the seedlings were incubated at 37°C. The harvesting periods were the same as in the alkaloid treatments. Total RNA was extracted from the whole seedlings with the RNeasy Plant Mini Kit (Qiagen, Tokyo, Japan). Total RNA (0.5 μg) was subjected to reverse transcription (45°C for 30 min), and then underwent PCR with cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s (24 to 40 cycles were performed for *HSP17.6C-CI*, 18 to 30 cycles for *HSP70*, 18 to 32 cycles for *HSP90.1*, and 18 to 24 cycles for *ACTIN-2*). The primers were

5'-CTCTAATTCCAAGCATC-3' (sense) and 5'-CCAGAGATATCAATGGAC-3' (antisense) for *HSP17.6C-CI*, 5'-GATCTCTAATAATGGCGGG-3' (sense) and 5'-CCAGTTTCAGAGTGACATAG-3' (antisense) for *HSP70*, 5'-CATCAACACGTTCTACAGC-3' (sense) and 5'-GTTCCAGACCTCGCAATGG-3' (antisense) for *HSP90.1*, and 5'-ACCTTGCTGGACGTGACCTTACTGAT-3' (sense) and 5'-GTTGTCTCGTGGATTCCAGCAGCTT-3' (antisense) for *ACTIN-2*. The amplified products were analyzed by 1% agarose gel electrophoresis. The band intensity was measured using NIH Image software (<http://rsbweb.nih.gov/nih-image/>) to determine the cycle where the PCR product was logarithmically amplified. The relative amounts of transcripts were calculated by standardizing the band intensities at the zero time point in each graph.

Statistical analysis

Data for *P* values were analyzed by Student's *t* test at a significance level of 0.05.

Results and discussion

Heat-tolerance screening

We listed the plant alkaloids that showed antimicrobial activities. Although there are a large number of antimicrobial alkaloids, we randomly chose commercially available ones from each major subgroup of natural alkaloids. Berberine and sanguinarine were the isoquinoline alkaloids, reserpine and yohimbine were the indole alkaloids, caffeine was the purine alkaloid, quinine was the quinoline alkaloid, and trigonelline was the pyridine alkaloid. These alkaloids have been reported to have antimicrobial activities (Cowan 1999; Godowski 1989; Almeida et al. 2006; Özçelik et al. 2011). Two antimicrobial alkaloids which do not belong to the major subgroups (capsaicin and colchicine) were also chosen (Jones et al. 1997; Cowan 1999). Because sanguinarine was found during the screening to show heat-tolerance-enhancing activity, we added other isoquinoline alkaloids including emetine, noscapine, and papaverine to the test list. Table 1 shows the 12 alkaloids and GDA as a positive control with their typical activities. Their structures are shown in Supplemental Fig. 1.

We designed a heat-tolerance screening system using sterile *Arabidopsis* seedlings. The optimum heat shock condition (at 47°C for 1 h) was determined by changing the incubation temperature from 45 to 50°C. We first tested GDA, the positive control for the heat-tolerance-enhancing effect, in order to validate the efficiency of the heat-tolerance

screening (Fig. 1a). When non-GDA-treated *Arabidopsis* seedlings were not exposed to heat, the fresh weights of the seedlings increased from 7 DAG (preHS) to 10 DAG (0 μ M, white bar). However, the fresh weights of *Arabidopsis* seedlings subjected to heat shock at 10 DAG (0 μ M, black bar) were lower than the fresh weights of the seedlings at 7 DAG, indicating that the seedlings were damaged by the heat shock. The addition of GDA at 50 μ M ameliorated the damage to the seedlings (50 μ M, black bar). This GDA concentration was the same as the effective concentration described in a previous report (Yamada et al., 2007). This suggests that our screening system worked as expected.

First, nine antimicrobial alkaloids, i.e., berberine, sanguinarine, reserpine, yohimbine, caffeine, quinine, trigonelline, capsaicin, and colchicine, were investigated in the heat-tolerance screening. Except for sanguinarine, their concentrations were 0, 0.5, 5, 50, and 500 μ M. The sanguinarine concentrations were 0, 0.05, 0.5, 5, and 50 μ M. As a result, we found that only sanguinarine showed heat-tolerance-enhancing effect among the alkaloids; another isoquinoline alkaloid, berberine, did not show such activity. We subjected three additional isoquinoline alkaloids (emetine, noscapine, and papaverine) to the screening and found that out of the five isoquinoline alkaloids tested, only sanguinarine showed heat-tolerance-enhancing activity (Table 1).

The dose-dependency of the heat-tolerance-enhancing effect of sanguinarine is shown in Fig. 1b. The fresh weight of 5 μ M sanguinarine-treated seedlings was significantly higher than that of non-treated seedlings after exposure to heat (Fig. 1b, black bars). On the other hand, treatment with berberine and papaverine did not alter the fresh weights of seedlings after the heat shock (Figs. 1c, d). Similar negative results were obtained when nine alkaloids including emetine, noscapine, yohimbine, reserpine, caffeine, quinine, trigonelline, capsaicin, and colchicine were tested.

Expression of heat shock protein genes by sanguinarine

It is known that the expression of HSP genes is related to heat tolerance in plants (Wang et al. 2004; Wahid et al. 2007; Zhang et al. 2014). GDA, which is a heat-tolerance enhancer, promotes some HSP genes in *Arabidopsis*. *HSP17.6C-CI*, *HSP70*, and *HSP90.1* are reported to be major GDA-inducible HSP genes (Yamada et al. 2007; Yoshida et al. 2011). This suggests that sanguinarine may also promote the expression of these three genes. Transcript accumulations of *HSP17.6C-CI*, *HSP70*, and *HSP90.1* in plants treated with sanguinarine, GDA, berberine, papaverine, and heat were analyzed. The *Actin-2* gene was tested as a housekeeping gene. The administration of sanguinarine, GDA, and heat promoted the expression of *HSP17.6C-CI*, *HSP70*, and *HSP90.1* (Figs. 2a, b, e). However, treatment with berberine and papaverine did not

change the transcript levels of *HSP17.6C-CI*, *HSP70*, or *HSP90.1* (Figs. 2c, d). *Actin-2* transcripts were not affected in any of the cases. These results indicate that sanguinarine promoted the expression of the HSP genes that were up-regulated by GDA and heat.

Proposed mechanism of sanguinarine

We searched for heat-tolerance enhancers among 12 well-known alkaloids that have highly diverse structures (Supplemental Fig. 1). Through the screening, we found that sanguinarine enhanced the heat tolerance of *Arabidopsis*. Sanguinarine, which is an alkaloid produced by Papaveraceae plants such as *Sanguinaria canadensis*, *Papaver somniferum*, *Bocconia frutescens*, *Chelidonium majus*, and *Macleaya cordata*, has been studied for possible medicinal applications. As a result, sanguinarine has been found to have diverse effects such as antihypertensive, antiplatelet, antimicrobial, and anticarcinogenic activities (Mackraj et al. 2008). However, there has been no report describing the physiological action of sanguinarine in plants.

Sanguinarine is the first true alkaloid which shows a heat-tolerance-enhancing effect in plants. Although several compounds, such as GDA (Yamada et al. 2007), benzyl alcohol (Saidi et al. 2005), salicylate, chlorophenols, celastrol (Saidi et al. 2007), and ITC (Hara et al. 2013) have been previously demonstrated to be heat-shock-response enhancers, GDA is the only compound whose target molecule, i.e., HSP90, has been determined. It was reported that part of the heat shock response is regulated by HSP90 (Yamada and Nishimura, 2008). Under normal conditions, HSF is inactivated by HSP90. GDA, after it is taken up into plant cells, may inhibit HSP90. This inhibition promotes the release of active HSF, and then the HSP genes are expressed. Sanguinarine enhanced the expressions of the three HSP genes (*HSP17.6C-CI*, *HSP70*, and *HSP90.1*) that were up-regulated by GDA. Interestingly, the time-course patterns of the gene expressions were similar between GDA and sanguinarine (Figs. 2a, b). These results suggest that sanguinarine might promote the heat shock response of *Arabidopsis* by a mechanism similar to that of GDA, although little structural commonality was found between the two compounds. Berberine, which possesses a benzyloquinoline structure, did not promote the heat shock response. This difference in their ability to promote the heat shock response of *Arabidopsis* may reveal structural differences between benzyloquinoline (berberine) and benzophenanthridine (sanguinarine). Although the structural formula of berberine is similar to that of sanguinarine (Supplemental Fig. 1), berberine and sanguinarine shows buckled and planar structures, respectively (Maiti and Kumar 2010). The difference in spatial structures may affect the activities for promoting the heat shock response.

Possible application of sanguinarine

In present agricultural practice, heat-tolerance breeding is a major method of maintaining crop productivity under extremely high temperatures (Wahid et al., 2007). However, efficient breeding methods remain to be established in many species. Alternative methods such as the application of heat-tolerance enhancers should be considered. Sanguinarine may be effective as a heat-tolerance enhancer. Pharmacological and toxicological studies have suggested that rigorous toxicity testing is needed before the application of this compound can be put into practice (Mackraj et al. 2008). Recently, sanguinarine has been applied to livestock production by expecting better performance (Kosina et al. 2004; Vieira et al. 2008; Yao et al. 2010). Sanguinarine may be found to be effective as a heat-tolerance enhancer in the fields of agriculture and environmental protection.

Author contributions MH and IK planned the research, performed the experiments, analyzed the data, and approved the manuscript.

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References

- Ainsworth EA, Ort DR (2010) How do we improve crop production in a warming world? *Plant Physiol* 154:526-530
- Allakhverdiev SI, Kreslavski VD, Klimov VV, Los DA, Carpentier R, Mohanty P (2008) Heat stress: an overview of molecular responses in photosynthesis. *Photosynth Res* 98:541-550
- Almeida AA, Farah A, Silva DA, Nunan EA, Glória MB (2006) Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. *J Agric Food Chem* 54:8738-8743
- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564-582
- Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H₂O₂ and catalase

276 during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant
 277 Physiol 116:1351-1357
 278
 279 Godowski KC (1989) Antimicrobial action of sanguinarine. J Clin Dent 1:96-101
 280
 281 Hall AE (2001) Crop Responses to Environment. CRC Press LLC, Boca Raton, Florida
 282
 283 Hara M, Harazaki A, Tabata K (2013) Administration of isothiocyanates enhances heat tolerance
 284 in *Arabidopsis thaliana*. Plant Growth Regul 69:71-77
 285
 286 Huang B, Xu C (2008) Identification and characterization of proteins associated with plant
 287 tolerance to heat stress. J Integr Plant Biol 50:1230-1237
 288
 289 Iba K (2002) Acclimative response to temperature stress in higher plants: approaches of gene
 290 engineering for temperature tolerance. Annu Rev Plant Biol 53:225-245
 291
 292 Jones NL, Shabib S, Sherman PM (1997) Capsaicin as an inhibitor of the growth of the gastric
 293 pathogen *Helicobacter pylori*. FEMS Microbiol Lett 146:223-227
 294
 295 Kosina P, Walterová D, Ulrichová J, Lichnovský V, Stiborová M, Rýdlová H, Vicar J, Krecman
 296 V, Brabec MJ, Simánek V (2004) Sanguinarine and chelerythrine: assessment of safety on pigs
 297 in ninety days feeding experiment. Food Chem Toxicol 42:85-91
 298
 299 Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf KD (2007) Complexity
 300 of the heat stress response in plants. Curr Opin Plant Biol 10:310-316
 301
 302 Mackraj I, Govender T, Gathiram P (2008) Sanguinarine. Cardiovasc Ther 26:75-83
 303
 304 Maiti M, Kumar GS (2010) Polymorphic nucleic acid binding of bioactive isoquinoline
 305 alkaloids and their role in cancer. J Nucleic Acids (doi: 10.4061/2010/593408)
 306
 307 Özçelik B, Kartal M, Orhan I (2011) Cytotoxicity, antiviral and antimicrobial activities of
 308 alkaloids, flavonoids, and phenolic acids. Pharm Biol 49:396-402
 309
 310 Roberts MF, Wink M (1998) Alkaloids: biochemistry, ecology, and medicinal applications.
 311 Plenum Press, New York

312

313 Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1999) Structural basis for
314 inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and
315 geldanamycin. J Med Chem 42:260-266

316

317 Ruelland E, Zachowski A (2010) How plants sense temperature. Environ Exp Bot 69:225-232

318

319 Saidi Y, Domini M, Choy F, Zryd JP, Schwitzguebel JP, Goloubinoff P (2007) Activation of the
320 heat shock response in plants by chlorophenols: transgenic *Physcomitrella patens* as a sensitive
321 biosensor for organic pollutants. Plant Cell Environ 30:753-763

322

323 Saidi Y, Finka A, Chakhporanian M, Zryd JP, Schaefer DG, Goloubinoff P (2005) Controlled
324 expression of recombinant proteins in *Physcomitrella patens* by a conditional heat-shock
325 promoter: a tool for plant research and biotechnology. Plant Mol Biol 59:697-711

326

327 Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from
328 stress: clues from transgenic plants. Plant Cell Environ 25:163-171

329

330 Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic
331 acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157-161

332

333 Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex
334 in cancer. Nat Rev Cancer 10:537-549

335

336 Vieira SL, Oyarzabal OA, Freitas DM, Berres J, Pena JEM, Torres CA, Coneglian JLB (2008)
337 Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic
338 acid. J Appl Poult Res 17:128–133

339

340 Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: An overview.
341 Environ Exp Bot 61:199-223

342

343 Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and
344 molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244-252

345

346 Waters ER (2013) The evolution, function, structure, and expression of the plant sHSPs. J Exp
347 Bot 64:391-403

- 348
- 349 Yamada K, Fukao Y, Hayashi M, Fukazawa M, Suzuki I, Nishimura M (2007) Cytosolic HSP90
350 regulates the heat shock response that is responsible for heat acclimation in *Arabidopsis*
351 *thaliana*. J Biol Chem 282:37794-37804
- 352
- 353 Yamada K, Nishimura M (2008) Cytosolic heat shock protein 90 regulates heat shock
354 transcription factor in *Arabidopsis thaliana*. Plant Signal Behav 3:660-662
- 355
- 356 Yao JY, Shen JY, Li XL, Xu Y, Hao GJ, Pan XY, Wang GX, Yin WL (2010) Effect of
357 sanguinarine from the leaves of *Macleaya cordata* against *Ichthyophthirius multifiliis* in grass
358 carp (*Ctenopharyngodon idella*). Parasitol Res 107:1035-1042
- 359
- 360 Yoshida T, Ohama N, Nakajima J, Kidokoro S, Mizoi J, Nakashima K, Maruyama K, Kim JM,
361 Seki M, Todaka D, Osakabe Y, Sakuma Y, Schöffl F, Shinozaki K, Yamaguchi-Shinozaki K
362 (2011) *Arabidopsis* HsfA1 transcription factors function as the main positive regulators in heat
363 shock-responsive gene expression. Mol Genet Genomics 286:321-332
- 364
- 365 Zhang L, Zhang Q, Gao Y, Pan H, Shi S, Wang Y (2014) Overexpression of heat shock protein
366 gene PfHSP21.4 in *Arabidopsis thaliana* enhances heat tolerance. Acta Physiol Plant
367 36:1555-1564

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369 **Conflict of Interest Statement**

370 The authors declare that they have no conflict of interest.

371

Table 1. Effects of natural alkaloids on heat tolerance of *Arabidopsis thaliana*

Classes and names	Typical activity	Heat tolerance enhancement (this study)*
Isoquinoline alkaloids		
Berberine	Antimicrobial, carcinostatic	No
Emetine	Antiprotozoal	No
Noscapine	Antitussive	No
Papaverine	Antispasmodic	No
Sanguinarine	Antimicrobial, carcinostatic	Yes
Indole alkaloids		
Reserpine	Antimicrobial, antipsychotic	No
Yohimbine	Antimicrobial, stimulant	No
Purine alkaloid		
Caffeine	Antimicrobial, stimulant	No
Quinoline alkaloid		
Quinine	Antimalarial, antimicrobial	No
Pyridine alkaloid		
Trigonelline	Antimicrobial, hypoglycemic	No
Others		
Capsaicin	Antimicrobial, irritant	No
Colchicine	Antigout, antimicrobial	No
Positive control		
Geldanamycin	Antimicrobial, carcinostatic	Yes

*Positive (Yes) and negative (No) judgments of the heat-tolerance-enhancing effects were made on the basis of significant differences between the fresh weights of control plants and those of compound-treated plants 3 days after heat shock.

Test concentrations were 0.05, 0.5, 5, and 50 μM (sanguinarine) and 0.5, 5, 50, and 500 μM (other alkaloids).

Figure legends

Fig. 1. Effects of pre-treatment with the test compounds on the growth of *Arabidopsis* seedlings after heat shock. The scheme of the experimental schedule is shown at the top. The fresh weights of the seedlings are shown. Geldanamycin (a), sanguinarine (b), berberine (c), and papaverine (d) were tested. The fresh weights of the seedlings just before the heat shock at 7 DAG are labeled preHS. White and black bars represent plants not exposed to heat and plants exposed to heat, respectively. Values and bars represent means $\pm$ SD (n=12). Asterisks show significant differences ($p < 0.05$) as determined by Student's *t*-test in a comparison between no treatment (0 μ M) and the compound treatments.

Fig. 2. Effects of test compounds and heat on the expression of *HSP* genes in *Arabidopsis* seedlings. Sanguinarine (a), geldanamycin (b), berberine (c), and papaverine (d) were tested. Heat (e) was a positive control. Four genes (*HSP17.6C-CI*, *HSP70*, *HSP90.1*, and *ACTIN-2*) were analyzed. The relative accumulation of transcripts was determined by RT-PCR. Values of the 0 h controls were standardized. Values and bars represent means $\pm$ SD (n=3). Asterisks show significant differences ($p < 0.05$) as determined by Student's *t*-test in a comparison between no incubation (0 h) and incubation.

Fig. 1 Hara and Kurita

Fig. 2 Hara and Kurita

Supplemental Fig. 1. Structures of alkaloids and geldanamycin tested in this study. Data were obtained from the web site of KEGG (<http://www.genome.jp/kegg/kegg2.html>). Sanguinarine and geldanamycin, which showed heat-tolerance-enhancing effects in our experiments, are highlighted by red frames.

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