

## Analysis of stress proteins at four different developmental stages in field-grown rice, *Oryza sativa* L. (cv. Pusa 169), plants

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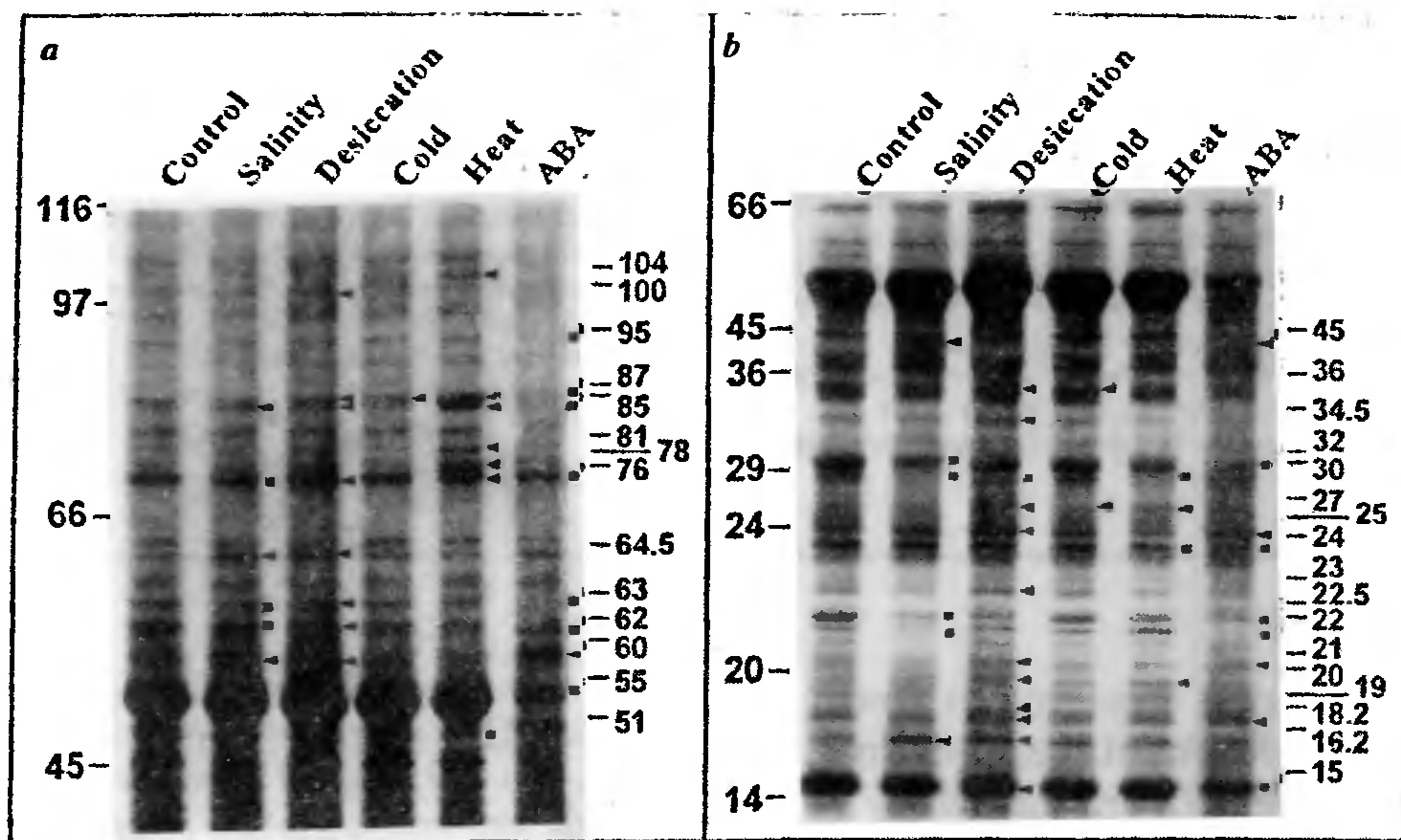
Grain yield in rice plant is adversely affected due to salinity, water stress, and low and high temperature stresses. These stress conditions affect the rice plants differentially at their different developmental stages. We have analysed alterations at cellular level of the various stress-associated proteins at four developmental stages in a high-yielding rice (cv. Pusa 169). These growth stages corresponded to 30-day-old (vegetative stage), 55-day-old (maximum tillering stage), 90-day-old (boat-leaf stage), and 110-day-old (seed-setting stage) plants. The sum total of polypeptide alterations was found to be different at the various growth stages analysed. While the alterations in the levels of some proteins were found to be common at the different stages (such as those with molecular weights of 30, 22.5, 22 kDa in response to salinity; 62, 60, 18.2 kDa in response to desiccation and 87 and 20 kDa in response to high temperature), alterations in levels of some other proteins showed growth stage-dependent difference. Elucidation of the precise identity of these proteins would possibly yield useful information on the response of rice plants to different abiotic stresses.

SEVERAL biotic and abiotic stress factors adversely affect cultivation of rice plants in various geographical locales<sup>1</sup>. The abiotic stresses result in more damage to growth and yield of rice plants compared to the biotic stress factors<sup>2</sup>. The major abiotic stress factors are: reduced

water availability (drought; DS), excess salinity (SS), and sub- (LS) and supra-optimal (HS) temperatures<sup>3</sup>. Rice plants are relatively more sensitive to SS at the seedling and the reproductive stages<sup>4</sup>. Excess of salts (primarily NaCl) leads to reduced seed germination and poor seedling vigour. While at the reproductive growth stage, high concentrations of salts adversely affect the number of spikelets formed per panicle<sup>5</sup>. The LS retards the development of rice seedlings, reduces tiller formation, and leads to poor fertility particularly reduced pollen viability<sup>6,7</sup>. In general, rice is adapted to regions of HS and prolonged sunshine<sup>3</sup>. However, certain growth stages of this crop are sensitive to HS, resulting in reduced tillering and height at vegetative stage, affecting adversely the panicle formation, and resulting in sterility and reduced grain filling<sup>7,8</sup>. DS affects cultivation of mainly the upland rices<sup>9</sup>. The extent of damage due to these stresses varies with the growth stage as well as the genotypic make-up of the rice plant<sup>7</sup>. In this communication, we report alterations in stress-associated proteins at four different developmental stages in a high-yielding Pusa 169 rice cultivar.

Field-grown plants of rice, *Oryza sativa* L. (cv. Pusa 169), were analysed in the present study. The plants were raised under standard agronomic practices in the field at the Indian Agricultural Research Institute, New Delhi. Since we could not subject the intact plants to different stresses because of the lack of controlled-conditioned phytotron facilities, the samples from plants raised under uninduced conditions (in the field) were subjected to various stresses under laboratory conditions. For this, the topmost leaf of the plant, in each case, was harvested and cut into 0.5–1 cm segments which were floated on distilled water (or any other solution, as desired in case of SS and abscisic acid). The stages analysed corresponded to 30-day-old (vegetative stage, stage A), 55-day-old (maximum tillering stage, stage B), 90-day-old (boat-leaf stage, stage C), and 110-day-old plants (seed-setting stage, stage D). The details of SS,

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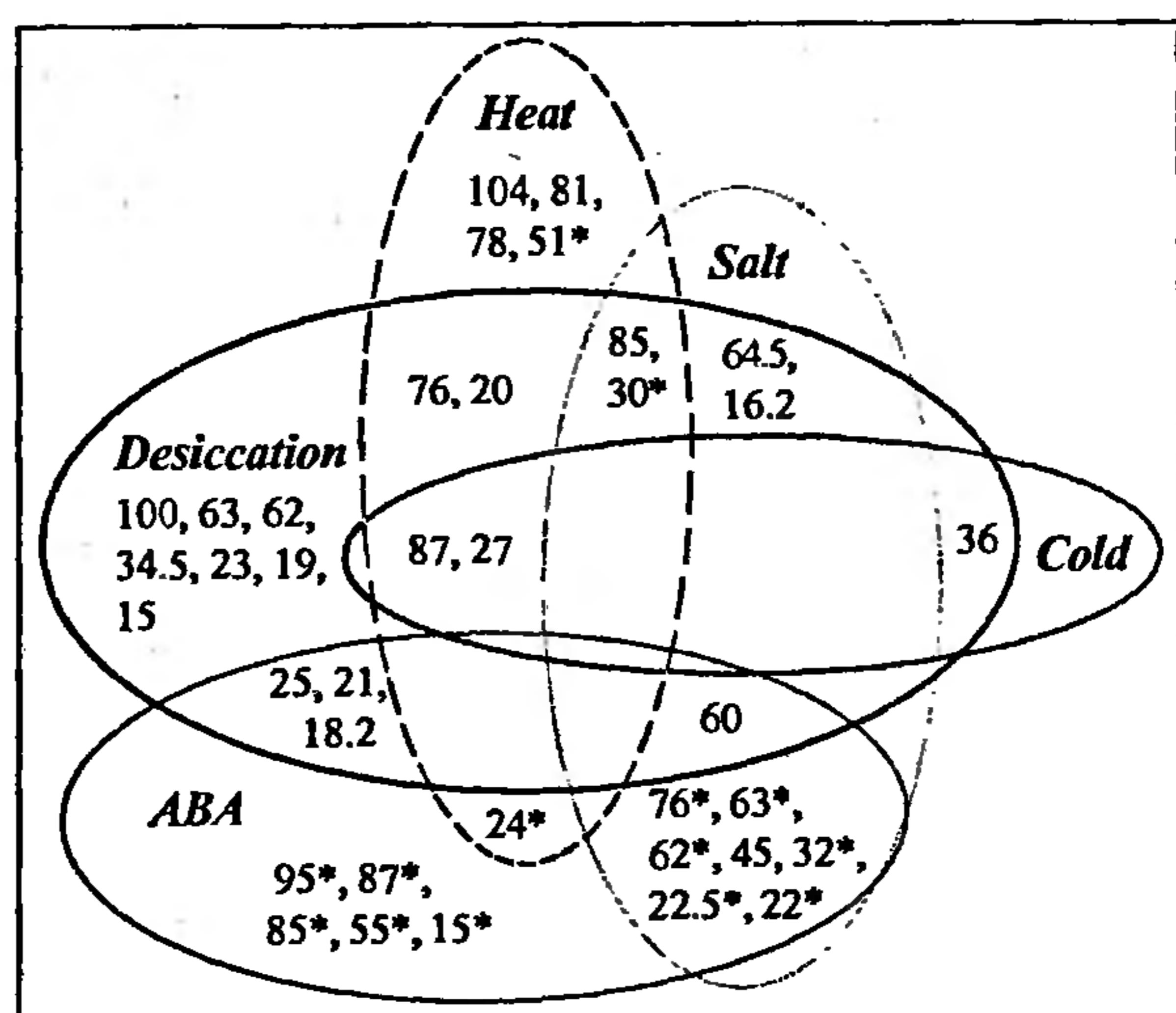


**Figure 1.** *a*, Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 30-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS: 5  $\pm$  2°C, 96 h; HS: 45°C, 6 h; and ABA: 10<sup>-4</sup> M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase, while those marked with a box show a decline). *b*, Analyses of low-molecular weight proteins as revealed by 15–22% linear gradient SDS polyacrylamide-gel. The other details are same as in the panel *a*.

DS, LS, HS, and ABA treatments, protein extraction and analysis were similar to those employed for analysis of Pusa 169 seedlings<sup>10–12</sup>.

It has been often observed that the response of a plant to a given stress varies with the different growth stages, i.e. a stress-tolerant plant is not necessarily tolerant at all the growth stages, and likewise, a stress-sensitive plant is not necessarily sensitive at all the growth stages<sup>7,13</sup>. Such observations are based on certain physiological/biochemical parameters such as reduction in growth rate and perturbations in metabolic activities including specific activities of different enzymes<sup>7,13</sup>. In this study, we have made attempts to highlight how the stress-induced protein alterations (which represent products of altered gene expression patterns) vary with the growth stages. This analysis was carried out on cv. Pusa 169, a high-yielding rice type<sup>14</sup>, and the four growth stages analysed corresponded to day 30, day 55, day 90 and day 110 after sowing. It is important to note here that while the leaves were harvested from the field-grown uninduced plants, stress conditions were imposed on the excised leaves in the laboratory in this work.

Figures 1–8 show data on alterations in the steady-state protein profiles at the various stages analysed in response to application of different stresses. Figures 1, 3, 5 and 7 represent the prominent protein changes, and the



**Figure 2.** Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 30-day-old rice plants (cv. Pusa 169). This analysis is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration, and 15–22% linear acrylamide gradient SDS gels. Molecular weights (kDa) of individual stress proteins are shown; those marked with asterisk (\*) decline in response to various stresses, while others increase in response to the stress treatments.

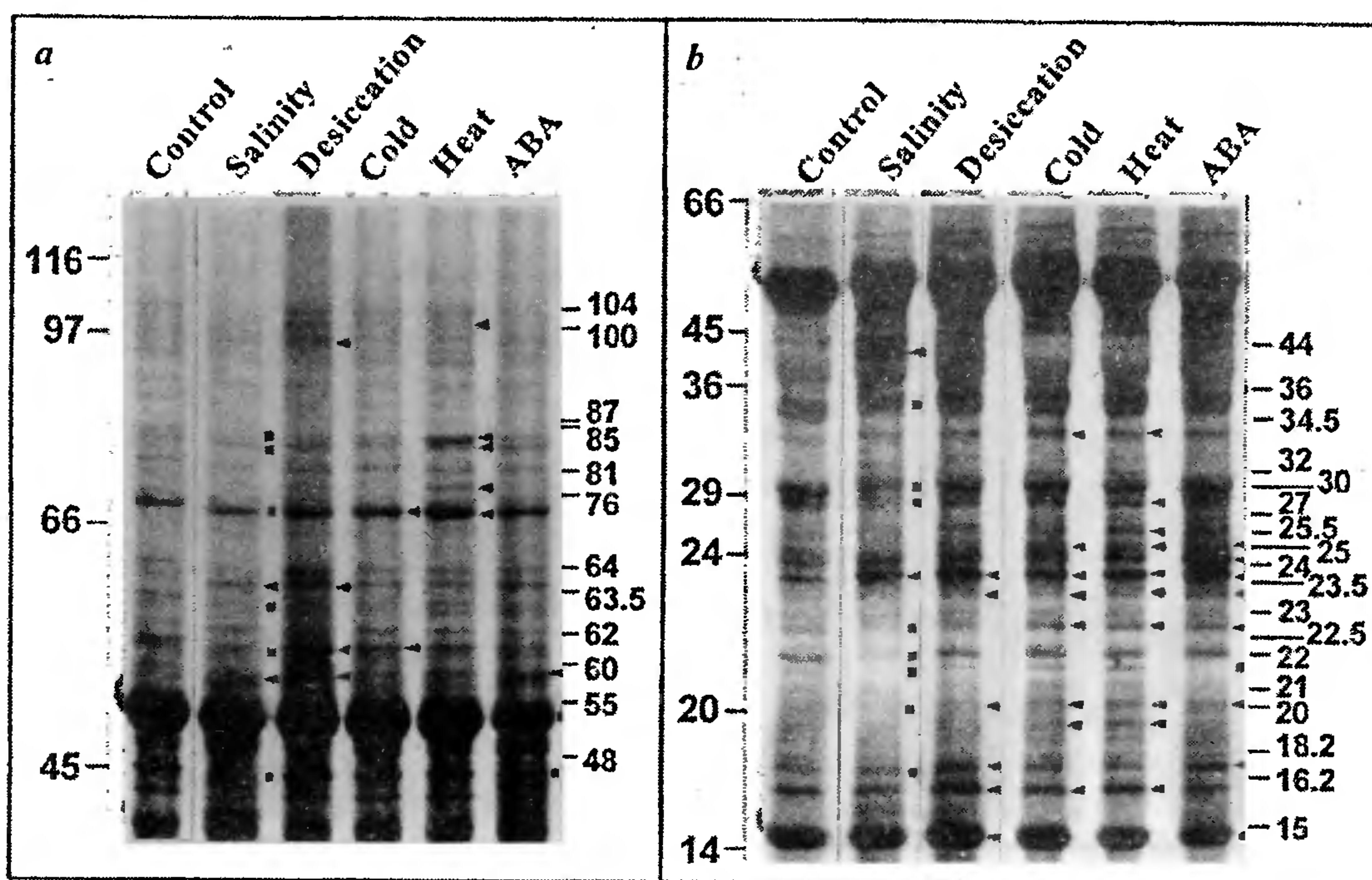


Figure 3. Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 55-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS:  $5 \pm 2^\circ\text{C}$ , 96 h; HS:  $45^\circ\text{C}$ , 6 h; and ABA:  $10^{-4}$  M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase), while those marked with a bar show a decline). *b*, Analyses of low-molecular weight proteins as revealed by 15-22% linear gradient SDS polyacrylamide-gel. The other details are same as in the panel *a*.

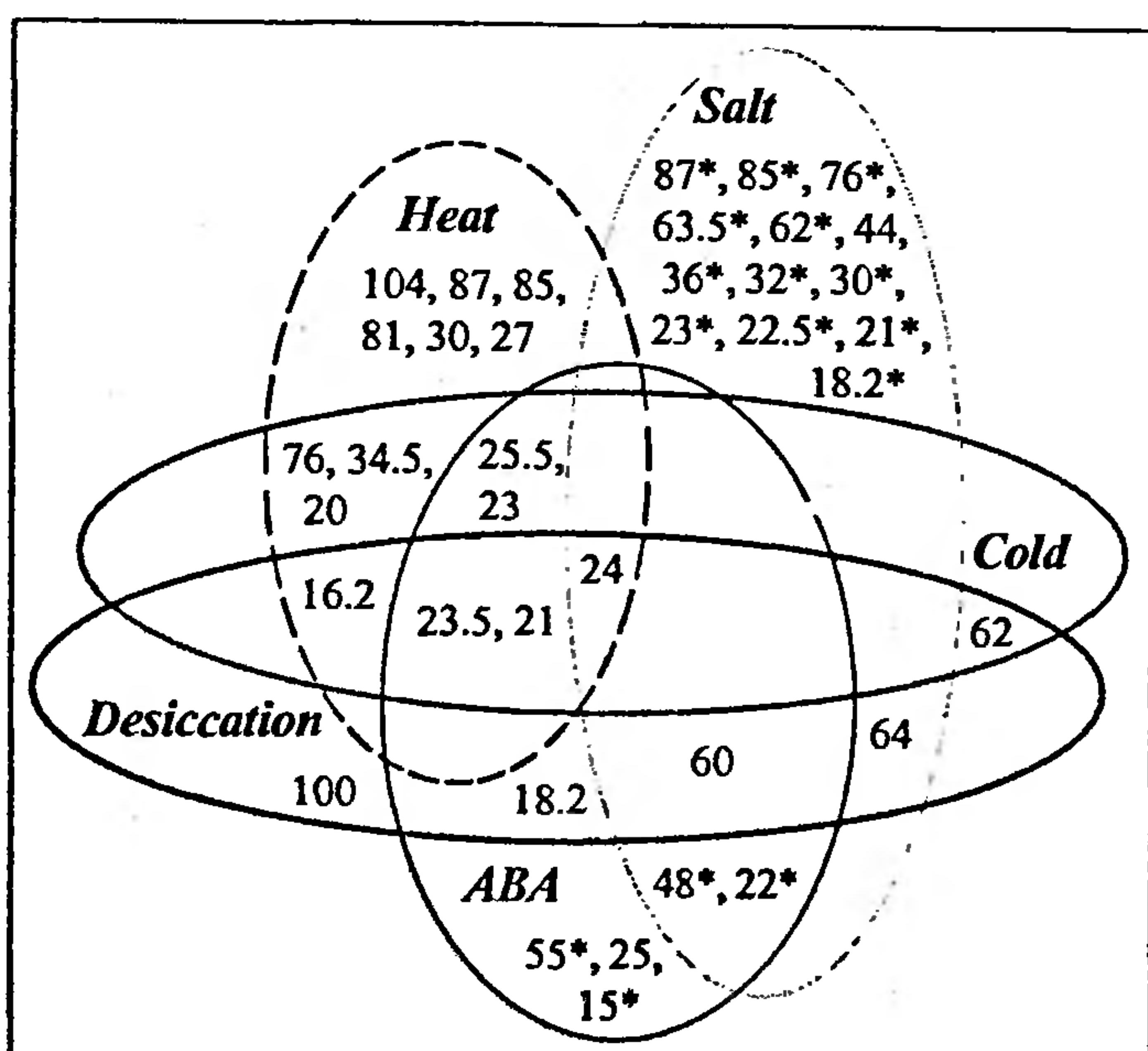
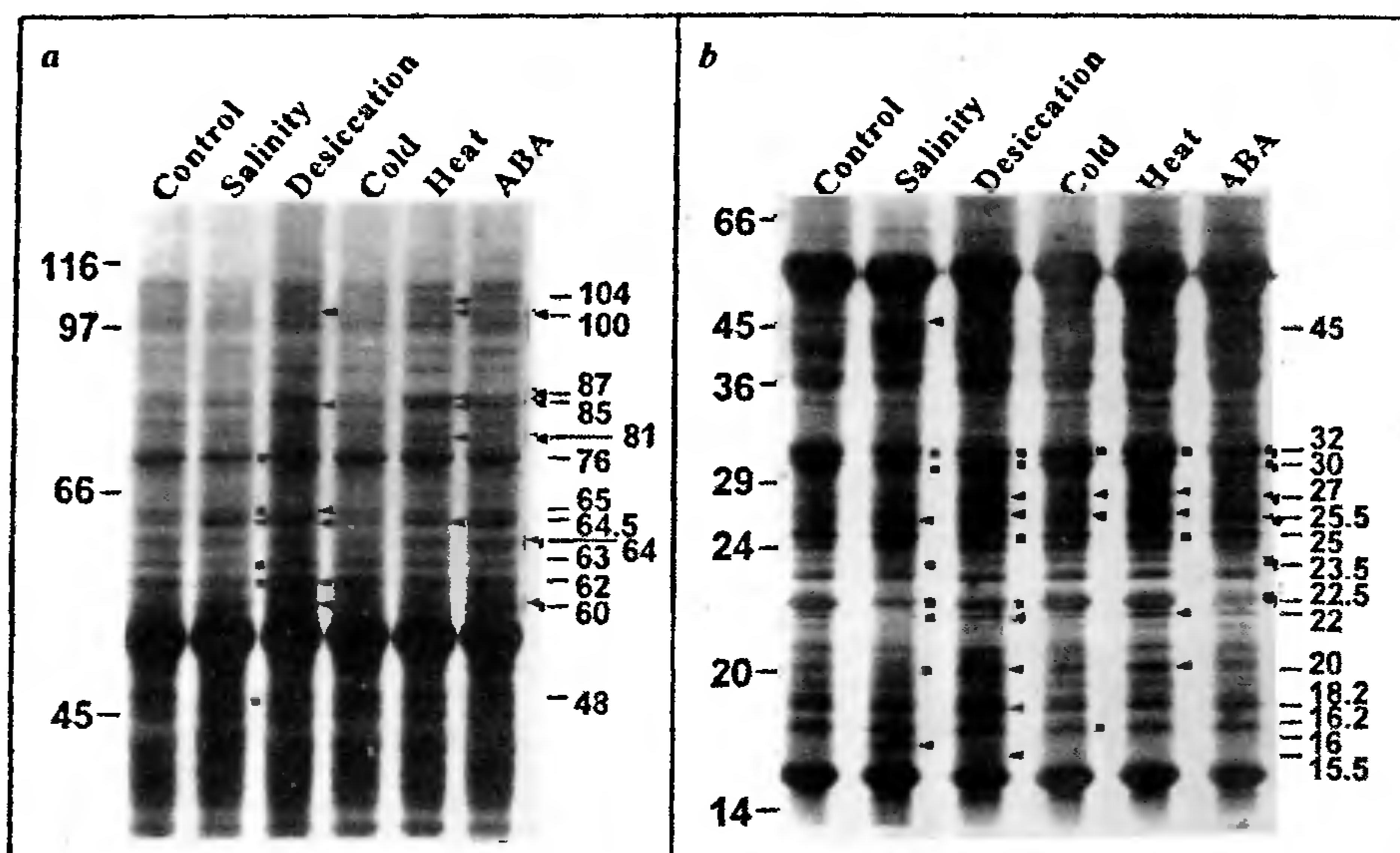
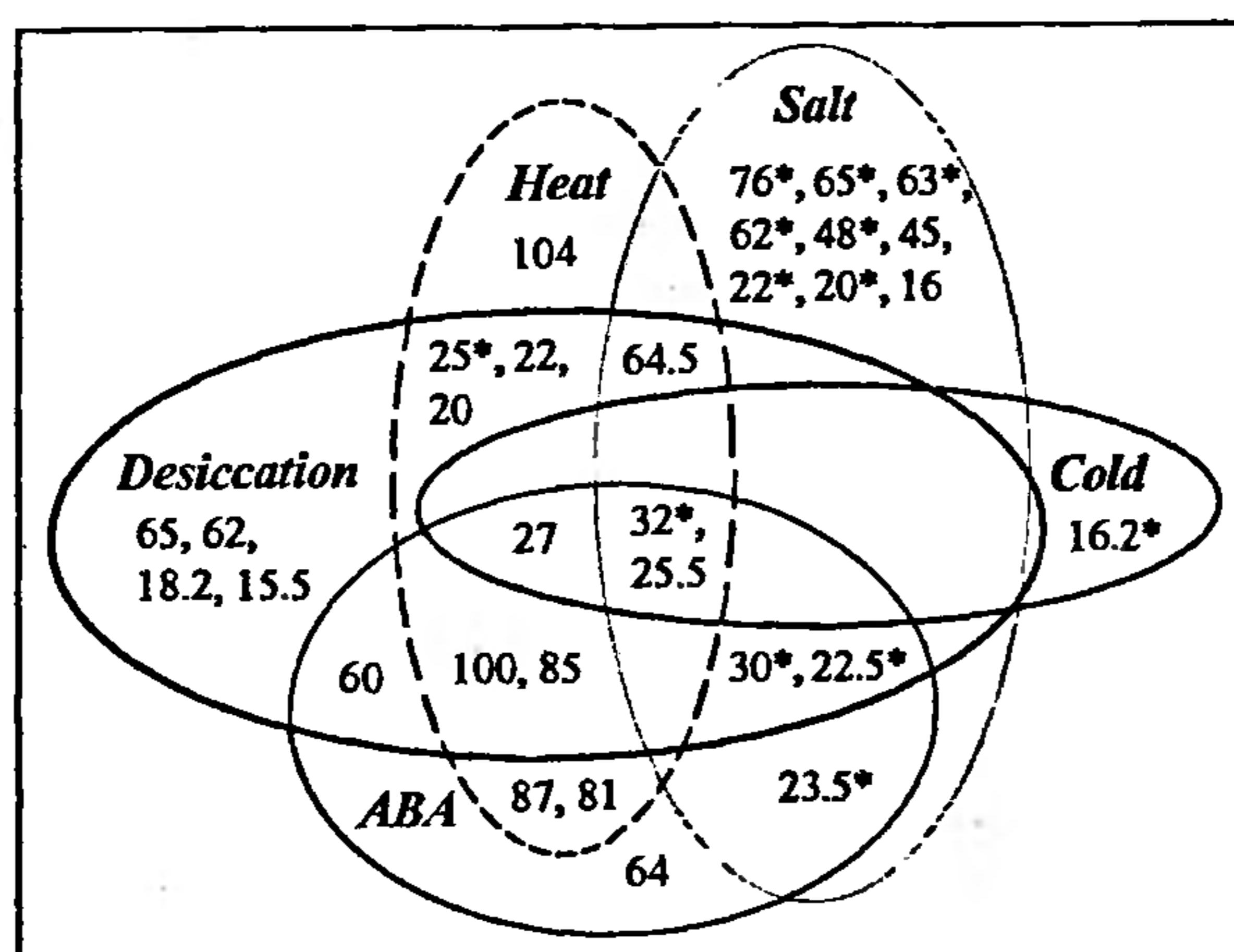


Figure 4. Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 55-day-old rice plants (cv. Pusa 169). This analysis is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration and 15-22% linear acrylamide gradient SDS gels. Molecular weight (kDa) of individual stress proteins is shown; those marked with asterisk (\*) decline in response to stresses while others increase in response to the stress treatments.

molecular weights of the protein alterations are shown on the right side of each panel in the figures. As is clear from these figures, the protein alterations ranged in molecular weight from as low as 15 kDa (in response to DS) to as high as 104 kDa (in response to HS). The results of this analysis have been summarized as Venn diagrams for each growth stage (Figures 2, 4, 6 and 8). In response to SS (200 mM NaCl, 96 h), 13 polypeptides showed alterations at the stage A, 18 at the stage B, 14 at the stage C, and 9 at the stage D. Importantly, alterations in certain specific polypeptides showed similar pattern in response to a given stress signal at all the stages. These include polypeptides with molecular weights of 30, 22.5, 22 kDa in response to SS; 62, 60, 18.2 kDa in response to DS; 87 and 20 kDa in response to HS; and 60 kDa in response to ABA application. However, no LS-responsive polypeptide was found conserved at the different stages analysed in this study. On the other hand, alterations in levels of certain specific polypeptides were found to be unique to a given stage of growth. For instance, accumulation of 64, 44, 24 and 16.2 kDa polypeptides in response to SS was noted only at the stage B. Further, specific polypeptide alterations were detected at either the early or the late stage of plant development but were not observed at all the stages. An example in this category includes a



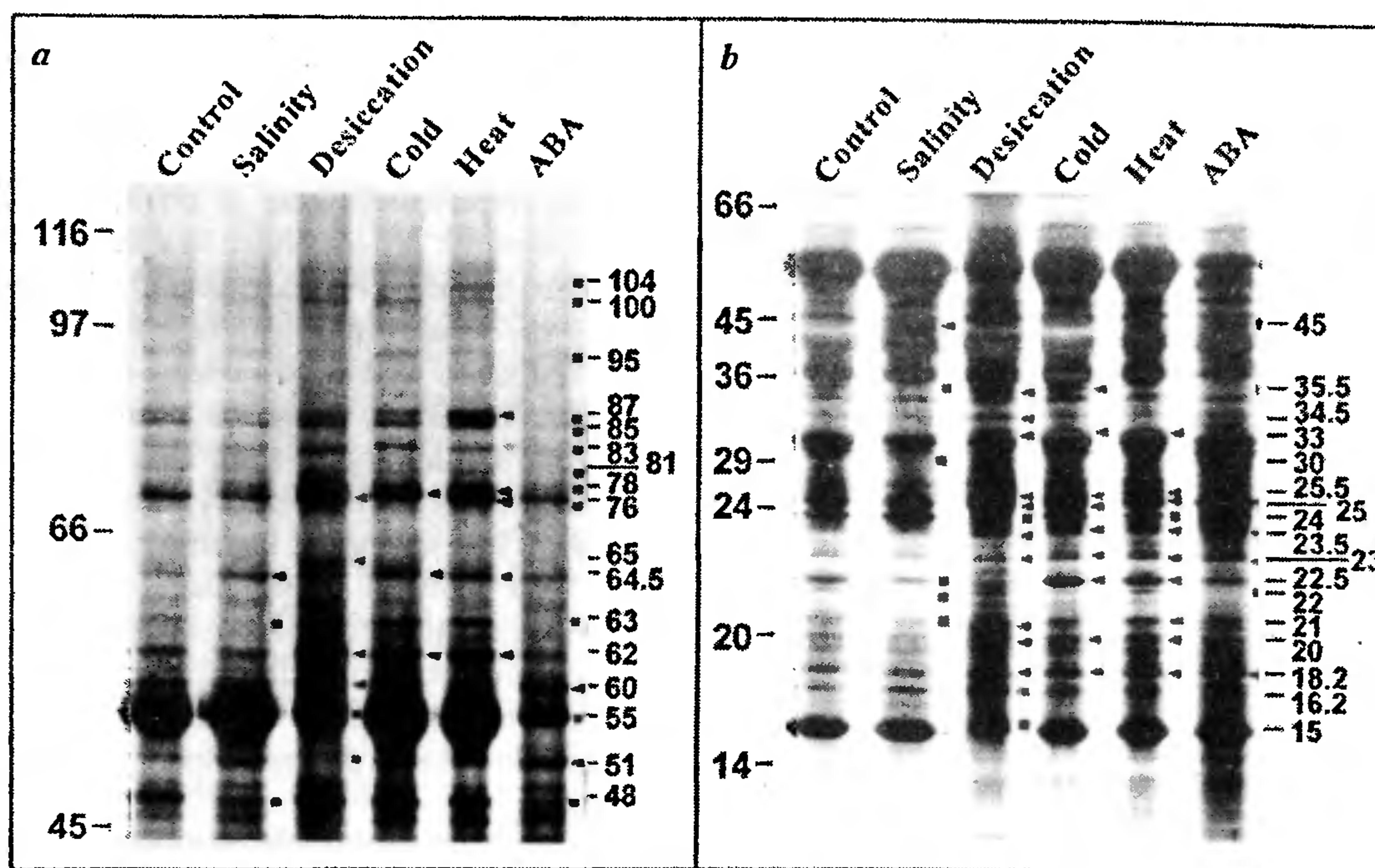
**Figure 5.** *a*, Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 90-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS: 5  $\pm$  2°C, 96 h; HS: 45°C, 6 h; and ABA: 10<sup>-4</sup> M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase, while those marked with ■ show a decline). *b*, Analyses of low-molecular weight proteins as revealed on 15-22 gradient SDS polyacrylamide-gel. The other details are same as in the panel *a*.



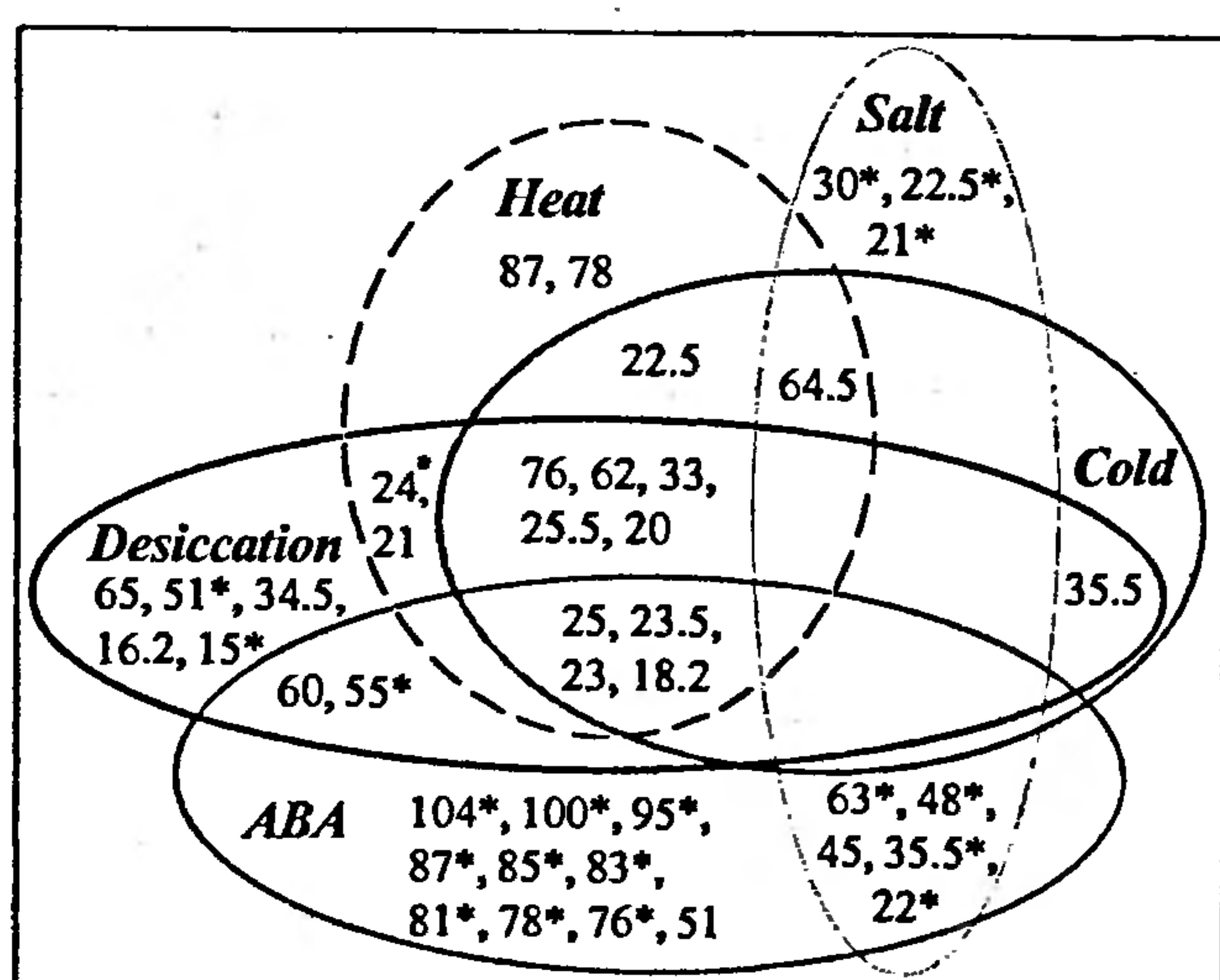
**Figure 6.** Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 90-day-old rice plants (cv. Pusa 169). This analysis is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration and 15-22% linear acrylamide gradient SDS gels. Molecular weight (kDa) of individual stress proteins is shown in the Figure, those marked with asterisk (\*) decline in response to stresses, while others increase in response to the stress treatments.

25.5 kDa polypeptide which accumulated in response to HS as well as LS at growth stages B, C and D but was not seen at growth stage A. On the other hand,

85 and 81 kDa polypeptides were accumulated at growth stages A, B and C but were not noted at growth stage D.



**Figure 7.** *a*, Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 110-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS:  $5 \pm 2^\circ\text{C}$ , 96 h; HS:  $45^\circ\text{C}$ , 6 h; and ABA:  $10^{-4}$  M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase, while those marked with a bar show a decline). *b*, Analyses of low-molecular weight proteins as revealed by 15-22% gradient SDS polyacrylamide-gel. The other details are same as in the panel *a*.



**Figure 8.** Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 110-day-old rice plants (cv. Pusa 169). This analyses is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration and 15-22% linear acrylamide gradient SDS gels. Molecular weight (kDa) of individual stress proteins is shown in the Figure; those marked with asterisk (\*) decline in response to stresses, while others increase in response to the stress treatments.

From the data presented, it is clear that stress-responsive alterations in protein pattern show a clear growth stage-independent as well as growth stage-

dependent response. Such studies may provide a lead for understanding the basis of developmental controls on stress-responsive gene expression patterns. Future studies must focus on the precise biochemical identity of various stress proteins. Once this information is available, it should be possible to work out the various components of the signal transduction cascades responsible for the altered patterns with respect to the developmental cues.

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