Structural evolution is the key to paternal identification in salt-resistant soybean breeding

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Application of paternal structural evolution identification in salt-tolerant soybean breeding

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Soybean breeding shows that soybean resources are increasingly narrow due to the depletion of ancestral varieties. We collected salt-tolerant wild soybean samples and seeds from 23 areas affected by heavy salt stress. SEM was used for structural evolution, Plant Print identification, salt and alkali resistance physiology, salt resistance gene and tissue culture experiments. We report here the discovery of Chinese wild soybean salt gland is first reported in the world. The salt tolerance of new soybean varieties is greatly improved by breeding wild soybean with salt gland as the male parent. A batch of new soybean varieties resistant to salt were obtained. The experiment revealed that the diversity of salt tolerance function of wild soybean was closely related to stress tolerance physiology, stress tolerance gene and phylogenetic structure evolution. Our results encourage the use of salt-resistant wild soybeans as paternal breeding to avoid undesirable breeding cycles at the expense of soybean ancestral varieties

The practice of soybean breeding shows that soybean resources are increasingly narrow due to the consumption of ancestral varieties. The effects of biodiversity loss are exacerbated over time[1]Wild soybean (*Glycine soja* Sieb. And Zucc.) is a product of adaptation to natural variation and selection[2-3]. More than 6000 germplasm resources of wild soybean were catalogued in China accounting for about 90% of the world total. It is necessary to Plant Print identification is required to identify whether salt-resistant wild soybean, the main contributor of salt-resistant wild soybean, can be used as a new resource for soybean breeding [4-5]. The Plant Print identification results of four soybean plants revealed that salt-resistant wild soybean had high homology with cultivated soybean, and genetic material was easy to exchang[6]. The results of gene identification showed that wild soybean and cultivated soybean had homology[7-12]. In 23 areas affected by heavy salt stress in China, we collected model samples of wild soybean that is resistant to salt stress.

The structure evolution of salt-tolerant wild soybean against salt stress was studied[13-14]. Our first report: " Discovery of wild soybean salt gland in China "[15]. Editorial Department of Chinese

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Science Bulletin reporting at home and abroad: A breakthrough has been made in the study of wild soybean salt gland in China [16]. Identification of a novel salt tolerance gene in wild soybean by whole-genome sequencing [17], identification of QTLs and functional genes involved in salt tolerance in soybean [18]. GsSnRK1 interplays with transcription factor GsERF7 from wild soybean to regulate soybean stress resistance, etc.,provides genetic characterization of genes for salt-resistant wild soybeans[19-21]. Here, we report results of the "PSPGT-China" experiment (PSPGT, Plant Print-identification-Salt resistant structure - evolution-Physiology-Gene-Tissue culture), that was established that was established in the China's severe salinization stress in 23 areas.

The characteristics of this experiment are in different distribution areas of salt-resistant wild soybean, i.e., the estuary of the Yellow River and the Songnen plain etc 23 area, and the experiment lasted for a long time (1998 to present) [22-69].

The salt-resistant wild soybean contrast experiment were implemented at two sites, (Site A : Baicheng district pH8.9 salinized soil and Site B: Changchun district pH7 black land) . Salt-resistant wild soybean grows in Site A: Baicheng district pH8.9 salinized soil, which have many salt glands are formed in the outer tangential part of its stem and leaf. However, wild soybean grows in Site B: Changchun district pH7 black land, which there are no salt glands formed in the outer tangential part of its stem and leaf.

Gene identification of salt-resistant wild soybean salt gland inherited a new variety of salt-resistant soybean

In this paper, scanning electron microscope (SEM) was used to observe the structural evolution of salt tolerance in wild soybean, namely salt gland formation and salt ion secretion function. The salt gland of wild soybean has a small stalk at the spherical base, which occurs at the intercellular space of the outer tangential wall of stem and leaf epidermis (Fig.1a-c). Salt gland layer by layer is formed and they vary in size. The head of salt gland is 21.6μm in diameter, and the stalk length is 1.2μm. When salt glands mature, they break up and release salt ion. The discovery that salt-resistant wild soybean has salt gland and can secrete salt ion. is reported for the first time at China and abroad (Fig.1). In order to verify that the salt-resistant structure of the father of salt-resistant wild soybean can be transferred to salt-resistant new soybean varieties, we conducted gene identification studies. One MYB transcription factor (GsMYB6-like) was cloned from both salt-resistant wild soybean plants and salt-resistant new varieties (Fig.1, d), with a full-length CDS of 796bp encoding 268 amino acids. Results Analysis showed high homology with transcription factor MYB6 (LOC100101858) located on chromosome 7 of the reference genome. Sequence analysis The Gm7 MYB6 encoded protein contained the SANT binding domain, namely, the MBY binding domain (Fig.1, d). When this gene was transformed into Arabidopsis thaliana after, Transgenic T3 seeds was found, which its isoflavone content was about 3 times higher than that of the control. Isoflavone and

anthocyanin are two important branches of plant brass synthesis. A homologous protein annotated as Anthocyanin Regulatory C1protein was discovered in wild soybean. From existing studies, it is speculated that the transcription suppressor Gm MYB6 is involved in the regulation of salt and alkali resistance and other stresses (Fig.1, e).

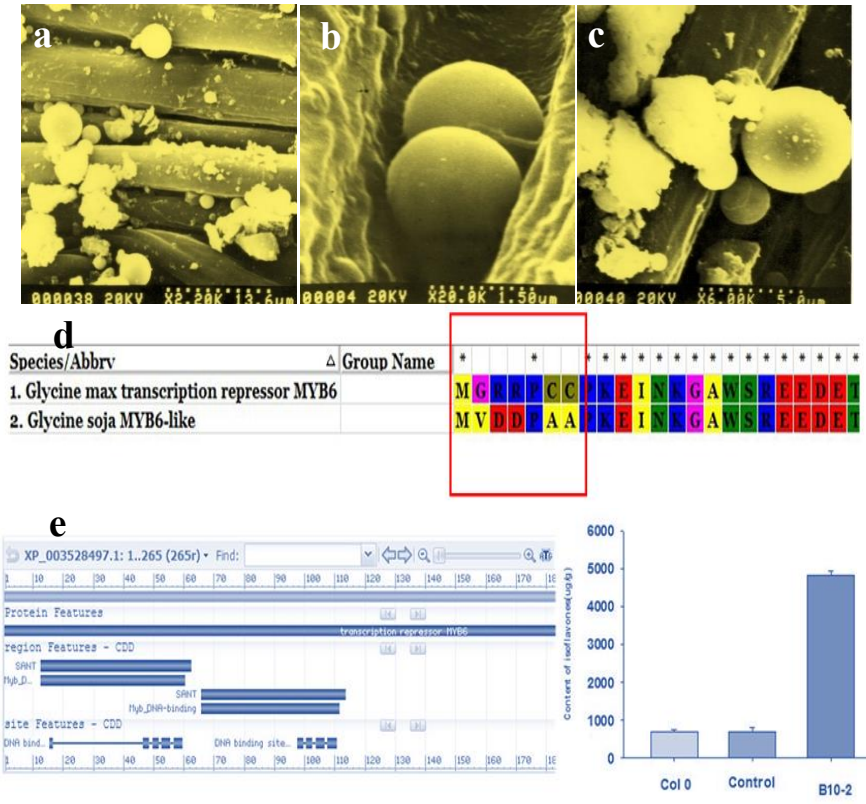

Fig.1|Genetic identification results Salt glands of salt-resistant wild soybean have been passed on to salt-resistant soybean varieties. a. Salt glands form layer after layer on the outer cutting wall of salt-tolerant wild soybean stem.
b, Wild soybean salt glands are distributed on the tangential wall of plant epidermal cells. The wild soybean salt gland consists of head cells and stem cells.
c,Mature salt glands of wild soybean plants broken, releasing triangular and conical salt grains to complete the salt removal process. Salt-resistant soybean breeding, the salt gland of wild soybean was inherited to salt-resistant soybean Jiyu 59.
d, Study on mutation sites of salt tolerance genes in new varieties of salt tolerance soybean and salt tolerance genes in wild soybean.
e, Changes of isoflavone content in salt-resistant soybean plants.

Structure evolution of vascular cylinder in roots of new soybean varieties under salt stress

According to the fossil record, the roots phylogeny is later than that of stems and leaves. The diarch of primary xylem of wild soybean root belongs to the primitive type (Fig.2a). The tetrarch and polyarch of root primary xylem belong to the evolutionary type. Breeding method of salt-resistant soybean by one pod transfer, which applied salt-resistant wild soybean as male parent and crossed with non-salt-resistant soybean female parent (Fig. 2b) obtain a salt-resistant soybean new variety its root evolution into triarch (Fig.2c). Another experiment is repeated, the root primary xylem of salt-resistant wild soybean is diarch (Fig.2d). It was hybridized with tetrarch (Fig. 2e), which is the primary xylem of the root of cultivated soybean mother parent. The primary xylem of root of new salt-resistant soybean varieties was also triarch (Fig. 2f). The breeding practice of salt-resistant soybean showed that the reduction of primary xylem archetypal in new salt-resistant soybean roots was an evolutionary characteristic of salt stress resistance, which could effectively reduce the volume of salt ions absorbed by roots. The result of this study is an important contribution to the research of salt resistant soybean breeding.

Salt resistant soybean breeding, the thylose of vessel elements was successfully transferred from wild soybean to salt-resistant soybean variety. There are three types of thylose, i.e. thylose cell type, thylose of rubber type, and thylose of crystalline type (Fig. 2g-i). Experimental results showed that thylose of cell type is formed by cell division after thin-walled cells enter from the pits on the side wall of vessel vlements. In vessel elements, multiple cell after nuclear division form a variety of cell type of thyloses (Fig.2g). Rubber type thylose is formed when secretory cells enter vessel elements (Fig.2h). Crystalline thylose is the single crystal thylose formed after parenchyma cells entered vessel elements and accumulated calcium oxalate in the vacuole (Fig.2i). Thylose can effectively prevent water and inorganic salt transport in vessel elements of salt-resistant soybean. Thylose also increased the hardness of vessel elements. Therefore, in the breeding process of salt-resistant soybean, different types of thylose came in vessel elements, which was the evolutionary feature of the structure of the antagonistic salt stress of salt-resistant soybean. The physiological study of salt tolerance revealed the effect of different salt stress concentration on the dry weight of root, stem and leaf of two cultivated soybeans, that is, the dry weight of no resistant to salt stress Zaofeng No. 5 was lower than that of resistant to salt stress Jiyu No. 59. That was proved that the salt resistant soybean Jiyu 59 had high salt resistance. (Fig.2j-k).

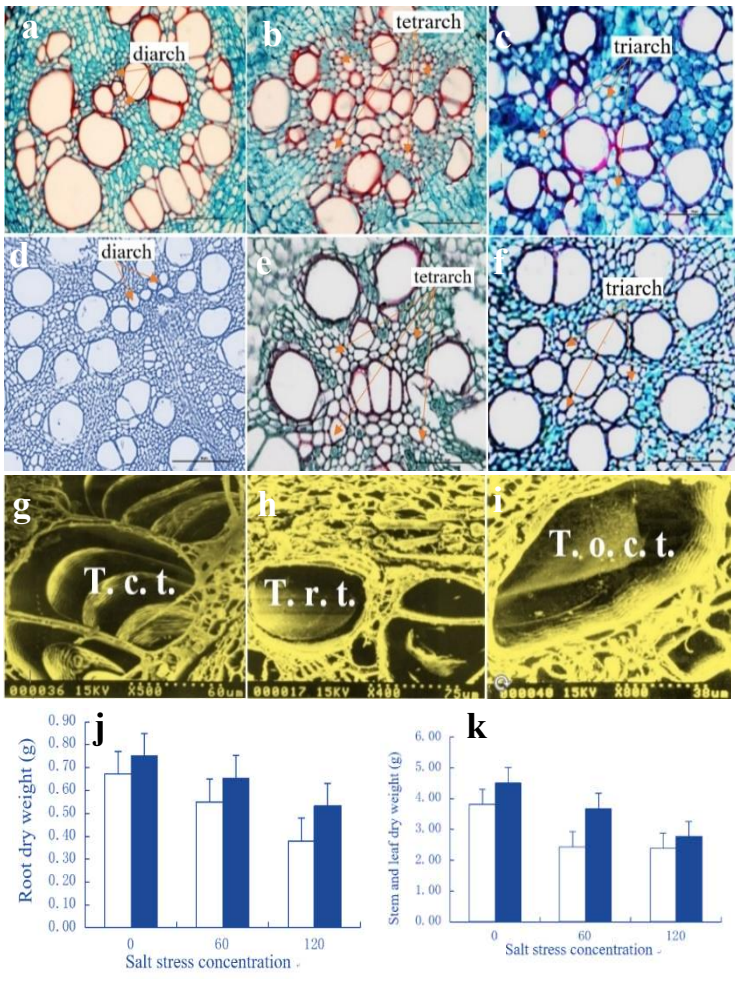

Fig.2| Structural evolution of salt-resistant soybean breeding roots. a, No. 001 salt-resistant wild soybean is as male parent its root primary xylem is diarch. **b,** No.1. salt-resistant soybean as female parent its root primary xylem is tetrarch**. c,** Salt-tolerant wild soybean root diarch was hybridized with cultivated soybean root tetrarch to obtain Jiyu No.59 salt-resistant soybean new varieties root primary xylem is triarch. It effectively reduces the volume of salt ions absorbed by the root. **d,** No. 002 male parent wild soybean primary xylem is a diarch. **e,** No.2 salt-resistant cultivate soybeans as female parent its root primary xylem is tetrarch. **f,** Xiaoli No.4 of new salt-resistant soybean variety was obtained by crossing the two. Xiaoli4 roots of this new salt-resistant soybean variety the primary xylem of are the triarch. It effectively reduces the volume of salt ions absorbed by the root. **g,** In the breeding process of salt-resistant soybean, thylose of salt-resistant wild soybean was successfully transferred to vessel element of salt-resistant new soybean variety, Tylose of cells type. **h,** Tylose of rubber type. **i,** Tylose of crystalline type. **Two dry matters of soybean comparison of dry matters. j,** Comparison two dry matter of soybean roots. **k,** Comparison of stem and leaf dry matter of two soybean species.

T. c. t. (Thylose cell type); **T. r. t.** (Thylose rubber type); **T. o. c. t.** (Thylose of crystal type).

Scale bars, 50μm (a-f). **SEM,** picture (g -i).

Structure evolution of root and stem of alkali resistant wild soybean under alkali stress

Compared with that under non-alkali stress（Fig.3a）. Under alkali stress, there was a thrombolic outer cortex in the most outer part of the root, with an average thickness of 13.99μm. The vascular cambium band consists of 2-3 layers of cells. The diameter of secondary xylem vessels was small, with an average diameter of 25.10μm. The vessel elements contains lye that has been stained purple and blue. The cambium between secondary xylem and phloem is weakened (Fig.3b). Compared with the non-alkali environment（Fig.3c). The number of vessel elements in the stem was significantly reduced（Fig.3d). which greatly reduced the volume of absorption and transport of alkali ions. It revealed the important biological characteristics of the evolution structure of wild soybean antagonistic alkali stress. Effects of different concentrations of alkali stress on proline content of wild soybean plants in two places. Under alkali stress, the proline content of Huinan non-alkali -resistant wild soybean and Tongyu alkali -resistant wild soybean increased by 78.98% and 57.01% respectively at the concentration of 90mM. The results showed that Tongyu wild soybean had strong alkali resistance（Fig.3e). Changes of net photosynthetic rate, stomatal conductance, intercellular carbon dioxide concentration and transpiration rate of wild soybean leaves in HuiNan and TongYu under different alkali stress concentrations. The P*n* of two kinds of wild soybean leaves increased slightly at the concentration of 60mM.However, the P*n* of the two soybean leaves showed a decreasing trend at the concentration of 90mM. Compared with the control, the P*n* of Huinan wild soybean leaf and Tongyu wild soybean leaf decreased by 82.18 and 44.94%, and the decrease of Huinan wild soybean leaf was much larger than that of Tongyu wild soybean (Fig.3d). Effects of different concentrations of alkali stress on proline content of wild soybean plants in two places (Fig.3d). Experiments have proved that salt-resistant wild soybean of Tutongyu has strong alkali resistance (Fig.3.f).

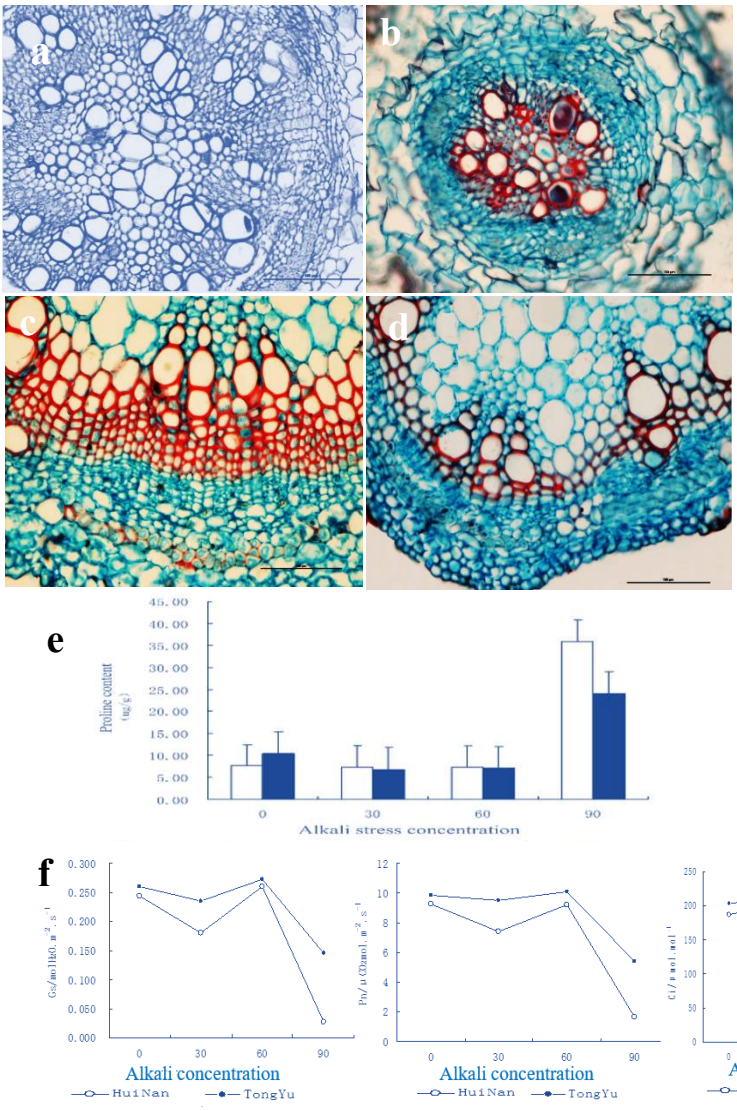

Fig.3 |The structure evolution pattern of the vascular cylinder in alkali-resistant wild soybean root and stem. a, Cross section of non-alkali stress wild soybean root, in Changchun district, Huinan, China. It showed that there were more vessel elements, and phloem. **b,** Structural evolution of alkali Stress root of wild soybean growing in alkali soil in Baicheng district, Tongyu, China, which results showed that the cortical cells were significantly reduced, the phloem sieve tubes and companion cells were reduced, the number of vessel elements in each vascular bundle was reduced. **c,** A cross section of non-alkali stress wild soybean stem were more vessel elements. **d,** Structural evolution of alkali stress stem, the number of vessel elements in each vascular bundle was reduced. But, the double phloem of vascular bundle was strong, the inner phloem sieve tubes and companion cells were increased and the myeloid cells were closely arranged, which increased alkali resistance.
Experiment on physiological stress resistance. e, Effects of different concentrations of alkali stress on proline content of wild soybean plants in two places. **f,** Changes of net photosynthetic rate, stomatal conductance, intercellular carbon dioxide concentration and transpiration rate of wild soybean leaves in Huinan and TongYu under different alkali stress concentrations. Experiments have proved that salt-resistant wild soybean of Tutongyu has strong alkali resistance.
Scale bars, 100μm (A, B, C, D).

Plant Print Concept

Plant Print refers to the apparent structure of a plant, including: stomatal complexes, guard cells, accessory guard cells, epidermal cells, salt glands, glandular hairs, vessel elements wall pores and other structures.

Plant Print and Finger Print are unique and exclusive. Plant Print also has structural stability. The results showed that Plant Print has important scientific properties that could be repeated and verified (Extended Data Fig. 1a-b).

We adopted the research method of David L. Dilcher, Academician, American Academy of Sciences, 1974, i.e. he classified the inlay of plant epidermal cells into type A-I.

Based on the research of David L. Delcher, Lu Jingmei has conducted studies on plant apparent structure identification for many years. Among hundreds of plants, Lu Jingmei found three new type of vertical wall inlay of epidermal cells of Plant Print.

The homology between wild soybean and cultivated soybean cotyledon nodes and seedlings is very high (Extended Data Fig. 1c-f).

Evolution of Plant Print

The identification experiment proved that the Plant Print characteristics of salt-resistant wild soybean and soybean were as follows: 2 guard cells are crescent, 2 auxiliary guard cells are parallel and unequal, and the radial wall of epidermal cells was type A（Extended Data Fig. 2, a-f). Experimental proof Wild soybean and soybean have evolved a high degree of homology, Their genetic material is easily communicated.

SEM identification, distinction between salt glands and glandular hairs of salt-resistant wild soybean

Salt gland and Glandular hair. **g,** Salt gland of wild soybean can secrete salt ions, which is colorless and odorless structure of secreting salt ions. **h,** Glandular hairs of wild soybean can secrete aromatic substances, which is the structure of aromatic substances secreted with pleasant taste. Through SEM experimental results, the problem that some people confuse the structure and function of salt gland and glandular hair was thoroughly clarified. SEM images provide first-hand experimental evidence for researchers to independently of salt glands and glandular hairs (Extended Data Fig. 2g-h).

Salt-resistant soybean breeding

The breeding of salt - resistant soybean was studied. Experiments proved that 95% of soybean flowers are self-pollination. By the time soybeans bloom, pollination and cleistogamy have been completed. The pollen of soybean plants germinates directly in the anther. The pollen tube carries two sperm through the anther wall, past the stigma, through the ovule, and grows towards the embryo sac (Extended Data Fig.5). A sperm fuses with an egg, forming a zygote and later developing into an embryo. The other sperm fuses with the two polar nuclei to form the endosperm nucleus, which develops into the endosperm: Nutrients from the endosperm are later absorbed by the cotyledons of the soybean embryo, which evolve into endosperm free soybean seeds, (Extended Data Fig.6). Three antipodal cells divide into multiple cells that provide nutrients for embryo development and then disappear. Soybean chromosome 2N was recovered after double fertilization. Soybean chromosome 2N was recovered after double fertilization.

In soybean self-pollination

The possibility of offspring variation is much less than cross-pollination. Therefore, in this study, one-pod transmission breeding of salt-resistant soybean was adopted (Extended Data Fig. 5a). Stamens of non-salt-resistant soybean mothers were removed.

Application pollen of salt-resistant wild soybean fathers was used for auxiliary pollination of the mothers (Extended Data Fig.5b-c). The results of salt-resistant soybean breeding experiments showed that the zygote divided into two cells, the base cell near the micropyle was larger, and the top cell far from the micropyle was smaller. The basal cell divides into a suspensor and the apical cell differentiates into a proembryo (Extended Data Fig. 5d). The suspensor cells and basal cells support the embryo body at the center of the embryo sac. Apical cells undergo transverse division, longitudinal division, and irregular division to form radicle, cotyledon, germ, and dicotyledon structures (Extended Data Fig.5e)

The results showed that the structure of salt-resistant soybean Jiyu59 embryo was larger than that of common soybean Zao feng embryo (Extended Data Fig. 5d, e, f).

The effects of salt stress on chlorophyll fluorescence in two cultivated soybean leaves were verified by physiological experiments (Extended Data Fig. 5g, h). Proved the new varieties of salt-

resistant soybeans of Jiyu No.59 salt - resistant soybean bility is very strong.

Salt-resistant wild soybean salt gland structure (Extended Data Fig. 5a-c) were effectively transferred to a series of salt-resistant soybean new varieties. New varieties of soybean resistant to salt cultivation have formed a complete evolutionary structure of salt glands rivaling salt stress. Mathematical statistics, showed that the salt gland of salt-resistant soybean was positively correlated with the antagonistic salt stress. Therefore, using possess salt gland salt-resistant wild soybean as the male parent use for salt-resistant soybean breeding, which they can effectively improve of a series of soybean new varieties salt-resistant ability, such as F_1, F_2.

The salt-resistant wild soybean with salt gland was used as the male parent

Non-salt-resistant cultivated soybean was used as the woody parent. A breeding program for salt-resistant soybeans has carried out （Extended Data Fig.6-7). The starch sheath of the father of salt-resistant wild soybean. The salt-tolerant wild soybean with salt-gland was used as the male parent and all the salt-tolerant new soybean varieties evolution salt-gland structure (Extended Data Fig.8a-h). The starch sheath of the father of salt-resistant wild soybean was successfully transcribed into A new salt-resistant soybean variety through genetic hybridization (Extended Data Fig. 8i-j). The heteromorphic vascular bundles in the male leaves of salt-resistant wild soybean (Extended Data Fig. 8k-l) were also successfully transcribed into the new salt-resistant soybean variety. Stress physiological experiment prove, the salt-resistant soybean new varieties have enhanced their salt-resistant ability, enhanced resistance to salt soybean new varieties rivaling the ability of adversity stress (Extended Data Fig.8m).

Accurately identify and screen the paternal resources of salt-resistant wild soybean, the structural evolution of four soybean species was studied from the outside to the inside

The four soybean types seed coat color, seed volume, hundred-grain weight and hilum color of wild soybean, semi-wild soybean, semi-cultivated soybean and cultivated soybean seeds are obviously different, so they can be used as auxiliary classification and identification indicators of soybean plants (Extended Data Fig.9a-d).

Experimental identification results show that differences in vegetative growth habit of four soybean types. Structure evolution of wild soybean is primitive and that of cultivated soybean is evolutionary (Extended Data Fig.9e-h).

The structural evolution identification of four soybean types was carried out to screen the fathers of salt-resistant wild soybean. Results showed that the structure evolution of wild soybean is primitive and that of cultivated soybean is evolutionary (Extended Data Fig. 9i-t).

The four types soybean structural evolution identification of segregation of the stem xylem. Sgregation results showed that the structure evolution of wild soybean is primitive and that of cultivated soybean is evolutionary (Extended Data Fig.9u-x).

Tissue culture experiment was carried out in order to rapidly propagate new soybean varieties resistant to salt

Soybean tissue culture is an aseptic operation to separate soybean cotyledons, i.e. explants, and

inoculate them into culture medium under artificially controlled conditions, such as nutrient solution, hormones, temperature, light, humidity, etc.

Germination process of salt-resistant soybean explants in tissue culture

Embryogenic cells produced by plant tissue culture are good receptors for genetic transformation, and the transformed embryogenic cells have a high frequency of plant regeneration. Through screening, transforming chimeras can be avoided and real transgenic plants can be obtained.

The results showed that the proportion of somatic embryos in soybean tissue culture was very high with proper control of culture conditions and 2,4-D concentration. The tissue culture structure could be used as the material for somatic hybridization, haploid breeding and transgenic research. The results of the study on the location, mode and process of plant regeneration in tissue culture have certain theoretical significance and the of salt-resistant soybean was expanded effectively practical application value (Extended Data Fig. 10, a-l).

The salt-tolerant wild soybean with salt gland was the male parent and crossed with the common soybean，which Some new varieties of salt-resistant soybean were obtained. It has formed a new mode of using biological technology to improve the ecological environment of saline-alkali soil and made efforts and contributions to the scientific and technological progress of soybean research in China (Extended Data Fig.10m-r).

Alkali resistant wild soybean structural evolution resistance to alkali stress experiment [22-28]. The effects of salt and stress reduction on photosynthesis [29-34] were positively correlated with the function of antagonizing salt stress. Including: stress physiological resistance to salt stress, alkali stress resistance. Cultivated soybean and wild soybean genome reveals the depth of heavy population structure and domestication[35], and half wild soybean genome reveals the complicated group structure weight and depth of infiltration[36], the influence of biodiversity[37], multidisciplinary dormancy gene in the process of domesticated crops parallel selection[38], crop genomics applications, and other research[39]. These experiments strongly support the selection of fathers in salt-resistant soybean breeding.About 6172 wild soybean germplasm resources[40] were catalogued in China, accounting for about 90% of the world total[41].The unique germplasm resources of salt-resistant wild soybean provide important natural resources for salt-resistant soybean breeding in China and the world[42-43].With respect to the origin of cultivated soybeans, the adaptive trajectory of soybeans from temperate to tropical origins was revealed by the study of the genetic complexity of different alleles regulating the LHY homolog J and E1[44,45]. Salt resistant soybean breedingThis study is based on the Chinese invention patent we obtained, i.e salt-resistant soybean Breeding method using wild soybean with salt gland as male parent. Number: ZL 200410. 011399.X. (2013) [46-54.]. In this study, tissue culture techniquewas used to expand the propagation rate and number of new salt-resistant soybean varieties[55].We selected salt-resistant wild soybeans with salt glands as the male parent and bred salt-resistant soybeans obtained a batch of new salt-resistant soybean varieties. Practice has

proved that new salt-resistant soybean varieties have A good harvest in saline soils with pH8.9 or higher, while non-salt-resistant soybeans die in the same ridge. Our new salt-resistant soybean varieties have been planted for export and farmers' orders. New salt-resistant soybean varieties have been applied and popularized in parts of the northeast China. The planting area of salt-resistant soybean varieties in Jilin Province has reached more than 79%. New varieties of salt-resistant soybeans have created an output value of 2.3 billion yuan[56]. Jilin Small grain No.4, won the gold Medal of the International Exposition[57]. Our new salt-resistant soybean varieties have been protected by The Ministry of Agriculture of China. promoted by The Salt-resistant and alkali-resistant varieties of Jilin Province [56-69]. Salt-resistant soybean breeding has obtained two first prizes of science and technology progress of jilin province [65-66]. Our results encourage the use of salt-resistant wild soybean as a paternal breeding strategy to avoid undesirable breeding cycles at the expense of soybean ancestral varieties.

Discussion

Salt-resistant wild soybean salt gland is the leader in the field of salt resistance of soybean plants.

Nevertheless, the directional (near vascular bundles) distribution of salt glands produces simultaneous structural evolution of continuous salt secretion. In this study, SEM was used to conduct Plant Print identification experiment to comprehensively characterize the structure of salt gland in epidermal cells.

The simultaneous structural evolution of millions of salt glands provided evidence for studying the development process of salt-resistant wild soybean. We found that the salt glands formed a reaction on the surface of the salt-resistant soybean, and planted and propagated the salt-resistant soybean for a long time without being reported, so that the structural evolution of the salt glands maintained the specificity of salt secretion.

Plant Print dynamic identification included several markers of salt ions released by effective mature salt gland fragmentation. In addition, the identity of salt glands in wild soybean has been controversial: we concluded that salt glands represent the structural evolution and function of salt secretion during plant phylogeny, and are exocrine structures that secrete colorless and odorless salt ions. To say that glandular hairs are salt glands is a momentary misrepresentation.Glandular hairs are exocrine structures secreting aromatic substances. Therefore, the morphological structure evolution and physiological function of salt glands and glandular hairs are obviously different, so they should not be confused.

In saline stress, salt glands of salt-resistant wild soybeans secrete salt effectively, by wrapping salt ions absorbed by the roots into millions of tiny salt-containing vacuoles through the keratinocytes of cortical cells, the salt is locally distributed in the cortex cells of wild soybean, which greatly reduced the toxicity of salt ions to cells. The salt-containing vacuole packets is transferred to the salt glandular head cells outside the stem and leaf, by transfer cells, and a set of closed salt-releasing processes and functional sides is completed.

We found that salt gland endocrine induces cortical cells to secrete keratinocytes to encapsulate salt ions to micro type development of exocrine salt glands. We describe a structural evolution of salt glands that can aggregate and secrete salt. The rich keratinocyte membrane of the endodermis secretes cells, which allows for local distribution of salt ions within the cell, eliminating salt injury and ensuring the type of salt gland that secretes salt continuously. In addition, salt glands were used as surface markers for the apparent structure of stems and leaves, and very pure salt gland clusters were sorted by SEM.

One of the unexpected findings of our analysis was the presence of salt glands in the outside tangential wall of wild soybean stems and leaves. We show that salt gland cells are closely related to transfer cells and have a different phylogenetic position from other cells. Both salt glands and transfer cells are derived from the late structure of plant evolution, so they conform to the structural evolution law of plant system from simple to complex.

It is reported that the salt gland of wild soybean has very rare primary salt-resistant function, which supports the natural existence of salt-resistant soybean breeding resources in China.

The structure evolution, Plant Print identification, stress physiology and gene cross identification of salt-resistant soybean breeding results were comprehensively analyzed. We found that salt-resistant wild soybean and cultivated soybean were closely related, with high homology and easy genetic material exchange. Salt-resistant wild soybean can effectively transfer salt-resistant structure such as salt gland, invasion body, starch sheath, irregular vascular bundle, flat vein mesophyle cells to the new salt-resistant soybean, and enhance the ability of the new salt-resistant soybean varieties to give rise to salt and alkali stress. New varieties of salt-resistant soybean have been widely promoted and applied in northeast China, with more than 79% planted in JiLin Province, which is very dry. It has received good economic benefits.

In this study, salt-resistant wild soybean with salt gland was used as the parent, which solved the problem of soybean self-pollination rate of 95%, and provided salt-resistant parent resources for the future development of salt-resistant soybean breeding in China. For example, we adopted the salt-resistant soybean breeding method of "passing from one pod", using salt glands on the body surface of new soybean varieties as the identification model. Promoted the guidance of salt-resistant soybean breeding activities.

Our salt-resistant gene identification demonstrated that although salt-resistant genes of salt glands are expressed in vitro, they represent a milestone in the formation of mature salt glands through interaction in vivo and in vitro. At the same time, further milestones of salt gland characteristics will come from structural evolution and epigenetic breeding identification and screening of paternal Plant Print.

Overall, we provide a comprehensive and detailed analysis of salt-resistant soybean varieties for human salt-resistant soybean breeding strategies.

This paper is the first to report the structural evolution of salt glands in wild soybean, and a comparative study of four soybean plants, including: Cotyledon node and seedling evolution,

macroscopic morphological evolution of asexual plants, the development and structural evolution of the micro embryo of sexual reproduction, the evolution of the microscopic three-section structure of stem, segregated vessel elements evolution and evolution of seed morphology and structure. Some new salt-resistant soybean variety have been obtained by combining study of structural evolution with Plant Print identification map, stress-resistant physiology and stress-resistant gene identification. Tissue culture has accelerated the propagation of new salt-resistant soybean varieties. All the experimental represents a necessary step on the road towards successful and safe necessary steps on the road to salt-resistant soybean breeding. Therefore, the undesirable cycle of salt-resistant soybean breeding at the expense of soybean ancestral varieties was avoided.

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Methods

We selected the mother cultivated soybean *Glycine max* (L.) Merr. They were provided by Soybean Research Center of Jilin Academy of Agricultural Sciences, which is pH 6.5-7.0. In N43°46′28.51″, E124°27′32.17. Salt-resistant cultivated soybean *Glycine max* (L.) Merr. were provided by Soybean Research Institute of Heilongjiang Academy of Agricultural Sciences. pH 8.2-8.9. In N 44°04′—46°40″, E 125°42′—130°10.

Salt-resistant wild soybeans *Glycine soja* Sieb.et Zucc. From different regions and different ecological environments were selected in this study. The male parent of salt-tolerant wild soybean was collected from salt stress area of China. In Heilongjiang Province, Daqing city, Qiqihar city, Zaodong county, Lindian county. In Jilin Province, Zhenlai county, Fuyu city, Qianguo county, Changling county, Nongan county, Baicheng city and Taonan city. In Liaoning Province, Zhangwu county, Xinmin city, Panjin county and Yingkou city. In Hebei Province, Xiong county and Xinan county. In the Inner Mongolia Autonomous Region‘s A-rong flags, Zhalantun, Zalaite flags and Ulanhot city. In Shandong Province, Kenli County and Dongying City. Plant specimens and seeds of salt-resistant wild soybean were collected from 23 highly salt-stressed soils with values ranging from pH 8.5 to 9.2.

We collected a lot of salt - resistant wild soybean seeds. After one year of field natural identification and laboratory macroscopic identification of salt stress test, 52 varieties of salt-resistant wild soybean paternal plant with obvious salt-resistant morphological structure were screened and confirmed.

SEM Drying method for identification of Plant Print of salt-resistant wild soybean samples. Plant Print sample containing water evaporates in vacuum or under normal temperature and pressure. When water molecules are completely detached from the sample surface, there is a surface tension between the sample surface and water, which is easy to damage the sample surface structure. Therefore, it is necessary to use a special method to eliminate the surface tension generated by the sample before identification, to remove the moisture inside the sample, so that the surface structure of the sample remains intact. There are two methods of salt-resistant wild soybean plant drying: one is natural drying method, the other is critical point drying method.

SEM Plant fracture method of salt-resistant wild soybean. To identify the internal structure of salt-resistant wild soybean cells, the cells and tissues must be effectively cut off, and the parts to be identified will be exposed. However, the fresh samples cut directly with a knife, which is easy to cause mechanical damage of the ultrastructure on the section and therefore cannot be identified. In order to maintain a good structure inside cells and tissues, samples can be randomly fractured by freezing or other external forces, the fractured samples can be cut again, treated with critical point drying, conducting treatment and identification.

SEM CO_2 critical point drying method for salt-resistant wild soybean plants. The liquid in the Plant Print sample is vaporized by using the characteristic that the surface tension is not produced in the critical point drying state, so as to ensure the complete drying of the Plant Print sample structure. This drying method avoids the influence of the surface tension on the Plant Print sample and better preserves the Plant Print sample. The original ultrastructure of Plant Print material will not be changed.

After dehydration and drying with ethanol of different gradient, the experimental material is stuck on the sample table. Gold plated film with B-3 ion sputtering, thickness 200 Å. The gilded test material of salt-resistant wild soybean was identified and photographed by Nissan S-570 scanning electron microscope with a voltage of 20kV.

Breeding One-pod transfer method to Salt tolerant soybean breeding. Salt-resistant wild soybean was used to make hybrid combination. Received hybrid, to the Hainan Province of increase the generation, in Gongzhuling planted F_2 generation. Generalized backcross combination was configured, and after receiving the hybrid, backcross for 1 generation was cultivated in Hainan. Backcross two generations were planted in Gongzhuling, and plants with salt glands were searched for. In autumn, the plants were selected by "one-pod breeding method". Backcross of 3-5 generations was cultivated in soybean research institute, and selection was made by genealogy method. The backcross 5 generations were analyzed and the strain selection was carried out. A comparative test of varieties was carried out in Gongzhuling. From 1996 to 1999, it participated in JiLin Provincial regional experiments. Participated in JiLin Provincial production test in 1999-2000.

Since 1996 to now, breeding One-pod transfer method to Salt tolerant soybean breeding has been using , Which have got a series of new salt-resistant soybean varieties have been successfully bred.

Wild soybean resources with salt glands were used as male parent, common cultivated soybean was used as female parent carrier, which they cross-fertilize to obtain soybean new varieties. i.e. No.3, No.4, No.5, No.98-1, No.9515 and No.59 etc. Successfully transfected a series of salt-resistant structures of salt-resistant wild soybean, including salt gland, salt-containing vacuole, transfer cells, thylose, special-shaped vascular bundle and flat vein mesophyll cells, into a new salt-resistant soybean variety. Enhanced the accuracy of salt-resistant soybean breeding, accelerated the breeding speed, shortened the breeding time, and broadened the germplasm resources of salt-resistant soybean breeding. This breeding method belongs to the pure natural soybean breeding, so it can effectively protect the safety of people eating soybean. New varieties of salt-resistant soybeans are favored by the international market, and China market.

The method of Gene identification of salt-tolerant wild soybean and salt-tolerant cultivated soybean new varieties. Seedlings of soybean were grown in the greenhouse under a photoperiod of 16 h light (100 l M photons m 2 sec1)/8 h dark at 25°C. Before stress treatments,2-week-old seedlings were carefully removed from the vermiculite, rinsed, and their roots were immersed in water for 2 days. For drought treatment, 2-week-old seedlings were subjected to 2.5% PEG or placed onto filter paper with 70% humidity at room temperature. For other treatments, seedlings were subjected to 200 mM NaCl, 100 l M ABA or 4°C for the indicated times. Quantitative PCR analysis. Total RNA was extracted from soybean seedlings or hairy roots using Trizol solution (Invitrogen, http://www.lifetechnologies. com), and first-strand cDNA was reverse-transcribed from 1 l g total RNA using Super Script TM III RNase H reverse transcriptase (Invitrogen). The cDNA fragments were amplified using gene-specific primers. The transcript level of TUBB1 was used as quantitative control. Real-time quantitative PCR was performed on a Roche (http://www.roche.com) LightCycler 480 II using a Thunderbird SYBR Green PCR kit. Samples with four replicates were quantified by the comparative cycle threshold method.

Transcription is the first step of protein biosynthesis, as well as the synthesis of tRNA and rRNA. Transcription is a process of synthesizing RNA strands under the catalysis of DNA-dependent RNA polymerase, which is formed by a single strand in DNA as template and free base as raw material. There are a lot of similarities or similarities with DNA replication.

Paraffin sectioning method was used to identify the structural evolution of salt-tolerant wild soybean. The method consists of 10 procedures, namely: (1) Draw materials; :(2) Immobilization; (3)Dehydration; (4)Lucency; (5)Waxdip; (6)Embedding; (7)ection; (8)Exhibition pieces; (9)Dewaxing and dyeing; (10)Film sealing and observation photography.

NIS-elements D 2.20 and SP2 (Build 243) image analysis software were used to measure the data of each Plant Print structural feature. Each group had 10 measurements and 5 replicates (50 times in total). The results of each experiment were the Mean value of the 5 replicates, and the results were indicated by Mean ± SD study.

Experimental methods and physiological index determination.

1) Determination of dry weight of roots, stems and leaves. In the morning of the second day after the last treatment, each pot of soybean was washed with running water and then washed with distilled water. After separating the roots and stems and leaves, the soybean was immediately dried at 105℃ for 15min, and then baked at 80℃ until constant weight, and weighed.

2) Determination of photosynthetic and chlorophyll fluorescence parameters. Li-6400 portable photosynthesis measurement system was adopted with a fixed red and blue light source, and the light intensity was 1200μmol·m-2·s-1.From 14:00 to 16:00 on the 10th day of treatment, the measurement site was the leaflets in the middle of the third compound leaves of the second compound leaf on the plant.The net photosynthetic rate (*Pn*), stomatal conductance (**Gs**), intercellular CO_2 concentration (*Ci*) and transpiration rate (*Tr*) of leaves were directly read by the instrument.

Li-6400 portable photosynthesis measuring system was adopted, and chlorophyll fluorescence head was replaced.After dark adaptation of the treated materials for two hours, the initial fluorescence (Fo) in the dark of the leaves was measured, and the maximum fluorescence (Fm) of the dark adapted leaves was measured by saturation flash, and Fv/Fm was read on the instrument.The initial fluorescence (Fo ') and maximum fluorescence (Fv ') of the leaves under light were measured by saturation flash. Fv '/Fm', ETR and qP were read from the instrument, and φ PSII (φ PSII=qP* Fv '/Fm') was calculated by formula.

3) Determination of chlorophyll content. Weigh 0.1-0.15g fresh leaves, cut them into pieces and put them into a centrifugal tube. Then add 10mL of the mixture of 80% acetone and anhydrous ethanol.Leave in the dark for more than 48 hours until chlorophyll is completely extracted.OD values at 663nm and 645nm were measured by uv spectrophotometer, and the total amount of chlorophyll was calculated by CT=20.29D645+8.05D663.

4) Determination of soluble sugar content. Anthrone reagent: 1.0g anthrone was weighed, dissolved in 1000ml 80% concentrated sulfuric acid, cooled to room temperature, stored in a brown bottle with a plug and stored in refrigerator.

Weigh the fresh sample 0.5-1.0g, cut it into pieces, mix it evenly, put it into a large test tube, add 15ml distilled water, boil it in a boiling water bath for 20min, take it out and cool it, filter it into a 100ml volumetric bottle, rinse the residue several times with distilled water, and keep the volume to scale.Take 1.0ml of the extracted solution of the sample to be tested plus 5ml of anthrone reagent, shake it well quickly, boil it in boiling water bath for 10min, take it out and cool it, measure THE OD value at 620nm wavelength with ultraviolet spectrophotometer, and calculate the soluble sugar content according to the standard curve.

5) Determination of proline content. 0.05g dry sample was weighed (depending on the test) and put into a centrifugal tube, then 10ml 3% sulfosalicylic acid was added. After sealing the bag and capping, it was extracted with boiling water for 30min. After cooling, it was centrifuged (3000r/min, 10min), and the supernatant was tested.

Content calculation. According to the absorption value of the sample solution, the corresponding proline concentration was found from the standard curve, and then substituted into the formula for calculation: proline content (umol/g W) =C*V/2/W/115, in the formula, C represents the proline concentration (ug/ mL) found from the standard curve;V represents the total volume of extracted liquid (mL);W represents the sampling amount (g);115 is the molecular weight of proline.

Data processing. Data processing and statistical analysis of variance were performed by statistical program SPSS17.0.All data are mean ± standard error of five replicates.LSD multiple comparison was used to compare the average value of each physiological index, at a significant level of 0.05, for multiple regression analysis.Excel is used for image analysis and processing.

Plant Print and Finger Print. They all have structural stability, exclusiveness and important scientific properties that could be repeated and verified. David L. Dilcher Academician of American Academy of Sciences, 1974, he classified the inset of plant epidermal cells wall type A-I.

Based on the research of David L. Delcher Academician, Lu Jingmei has conducted studies on plant apparent structure identification for many years. Among hundreds of plants, she found new type inset of epidermal wall cells of three Plant Print (Extended Data Fig. 1a-b).

Data processing method for soybean acquisition. Nis-elements D 2.20, SP2 (Build 243) image analysis software was used to measure the characteristic data of each structure. Each group had 10 measurements and 5 repeats (50 times in total). The experimental results were the Mean of 5 repeated experiments, and the results were indicated by Mean±SD study.

Stomatal density: is the number of stomata per mm^2. Stomatal index (I) was calculated by using the average cell number (E) and stomatal number (S) of the 10 fields. The calculation formula was: $I = [S/(E + S)] \times 100$.

Statistical analysis method. SPSS20.0 software was used for statistical analysis, and $P<0.05$ was considered as significant difference. Analysis of variance: One-way an OVA was used, multiple comparison was performed by LSD method or Tamhane, S method (when variance was not uniform). Correlation analysis: correlation analysis between multiple variables, the correlation between variables was measured by Pearson correlation coefficient; Distance analysis: Using Euclidean distance as the metric standard, dissimilarity matrix was constructed. Regression analysis: linear

regression, using the entry method to import variables; Multivariate linear regression, variables through the stepwise method into the model, the regression equation.

Statistical of Plant Print identification characteristics correlation analysis. According to Conover's reference standard of guard cell length, small(S)<12μm; Medium small (MS) = 12-19 microns; Medium (M) = 20 to 34 microns; Medium large (ML) = 35 to 42 microns; Large (L) = 43-65 microns; Very large (VL) > 65 microns. It can be concluded that the guard cell length grade of some plants of soybean genus is between MS and M, and the grades of wild, semi-wild, semi-cultivated and cultivated soybeans are successively increased (Table 1.).

Table 1. Correlation analysis unit of Plant Print characteristics of wild soybean: μm

	GL(μm)	GW(μm)	GP(μm)	GA(μm^2)	SP(μm)	SA(μm^2)	EP(μm)	EA(μm^2)
GL(μm)								
GW(μm)	-0.092							
GP(μm)	-0.293	0.318		**	**	**	**	**
GA(μm^2)	-0.305	0.153	0.731		**	**	**	**
SP(μm)	-0.083	0.044	0.703	0.767		**	**	**
SA(μm^2)	-0.093	0.133	0.701	0.786	0.969		**	**
EP(μm)	-0.082	0.089	0.753	0.753	0.880	0.824		**
EA(μm^2)	-0.111	0.116	0.753	0.868	0.938	0.940	0.892	

** Correlation is significant at the 0.01 level (2-tailed).

GL: length of guard cell; **GW:** guard cell width; **GP:** guard cell perimeter; **GA:** guard cell area; **SP:** perimeter of accessory cell; **SA:** area of accessory cell; **EP:** epidermal cell circumference; **EA:** epidermal cell area; **SI:** stomatal index; **SD:** stomatal density

Distance analysis. In order to analyze the relationship between the tested soybean plants, the dissimilarity matrix was established by using 11 characteristic parameters of Plant Print.The results of distance analysis showed that Plant Print relationship of wild soybean decreased in order of semi-wild soybean, semi-cultivated soybean and cultivated soybean under mesophytic environment. The Plant Print relationship between semi-wild soybean and wild soybean was closest, and the Plant Print relationship between semi-cultivated soybean and cultivated soybean was decreased successively. The Plant Print relationship between semi-cultivated soybean and semi-wild soybean is the furthest, and that between cultivated soybean and Plant Print is the closest. The cultivated soybean and semi-wild soybean Plant Print have the longest relationship, and the semi-cultivated soybean Plant Print has the closest relationship.

The data showed that the Plant Print characteristics of the apparent structure of wild soybean in salt-resistant environment were closely related to semi-cultivated soybean and cultivated soybean, but far related to Plant Print of semi-wild soybean.

Regression analysis. Based on the Plant Print characteristic parameters of leaf apparent structure of wild soybean 2013-001-01 (General elm), stepwise regression method was used to analyze the multiple linear regression of guard cell area Y to guard cell perimeter x1, guard cell length X2, guard

cell width x3.The analysis results showed that after removing the two independent variables corresponding to the insignificant partial regression coefficient: guard cell length (P=0.57 > 0.05) and guard cell width (P=0.62 > 0.05), the optimal equation was guard cell area y versus guard cell perimeter x1: y=-10.25+2.24 X1, R2=0.54.The regression equation of accessory cell area Y to accessory cell circumference X was established by unary linear regression: y=-449.79+8.24x, R2=0.94.The regression equation of epidermal cell area Y to epidermal cell perimeter x was established by unitary linear regression: y=24.97+6.13x, R2=0.80. the wild soybean 2013-001-01.

Plant Print identification of Salt tolerant wild and cultivated soybeans the mathematical statistics method

This Plant Print identification of the apparent structural characteristics of some soybean plants in Jilin Province. Proposed the theory that Plant Print can supplement and improve the classification characteristics of soybean plants by relying on the apparent structural characteristics of soybean plants' leaves. The experimental results proved that the Plant Print types of leaf apparent structure of tested soybean plants were all flat and unequal, and the pattern of vertical wall was mainly A-type, which proved the homology similarity of plants of the same genus.

2) Correlation analysis of plant Print characteristics of cultivated soybean during

The correlation coefficients between guard cell length and epidermal cell circumference and area were all less than 0, and all satisfied P < 0.01, indicating that there was a significant linear negative correlation between guard cell length and epidermal cell circumference and area. Guard cell perimeter and guard cell area; Peripheral cell circumference and peripheral cell area; The correlation coefficients of epidermal cell circumference and epidermal cell area were all greater than 0 and all satisfied P < 0.01, indicating a significant linear positive correlation between the two in the group. The correlation coefficients of guard cell width, guard cell perimeter, and guard cell area were all greater than 0, and all met P < 0.05, indicating that there was a significant linear positive correlation between the two groups (Table 2).

Table 2. Unit of correlation analysis of Plant Print characteristics of cultivated soybean: μm

	GL(μm)	GW(μm)	GP(μm)	GA(μm^2)	SP(μm)	SA(μm^2)	EP(μm)	EA(μm^2)
GL(μm)							**	**
GW(μm)	-0.161		*	*				
GP(μm)	0.011	0.452		**				
GA(μm^2)	-0.299	0.477	0.613					
SP(μm)	-0.006	-0.309	-0.081	0.12		**		
SA(μm^2)	-0.002	-0.296	-0.089	0.105	0.969			
EP(μm)	-0.615	-0.09	0.204	0.216	0.09	0.05		**
EA(μm^2)	-0.704	-0.117	0.002	0.252	0.014	-0.031	0.908	

**Correlation is significant at the 0.01 level (2-tailed).

GL: length of guard cell; **GW:** guard cell width; **GP:** guard cell perimeter; **GA:** guard cell area; **SP:** perimeter of accessory cell; **SA:** area of accessory cell; **EP:** epidermal cell circumference; **EA:** epidermal cell area; **SI:** stomatal index; **SD:** stomatal density.

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61.Jilin Jiyu 59 salt-resistant soybean has been confirmed the extension variety. Jilin Province variety Certification Committee (2001).

62.New Salt-Resistant Soybean Variety No. 4 obtain the certificate of plant variety right issued by *Ministry of Agriculture*. PRC (2002).

63.Jilin Province identified the salt-tolerant jilin Small Grain No. 4 salt-resistant soybean has been confirmed the extension variety. *Jilin Province variety Certification Committee* (2000).

64.New Salt-Resistant Soybean Variety No.3 obtain the certificate of plant variety right issued by *Ministry of Agriculture*. PRC (2002).

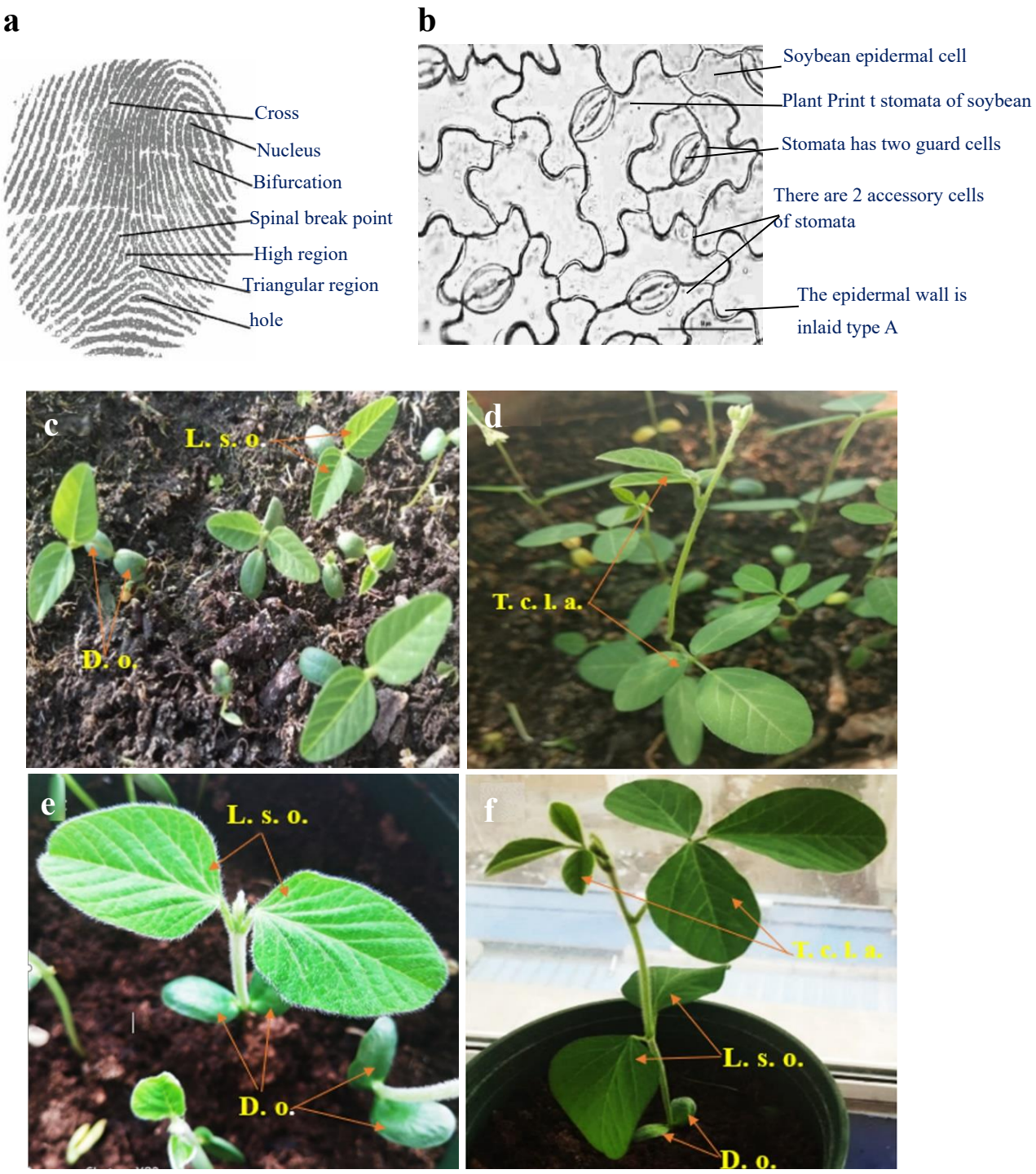
65.Anatomical study on salt resistance of wild soybean and its application in soybean breeding won the first prize of science and technology progress of *Jilin* province in (2004).

66.Research on Basic Biology of Wild Soybean in China and Its Application in Genetic Improvement, won the first prize of Science and Technology Progress of *Jilin Province* in(2000).

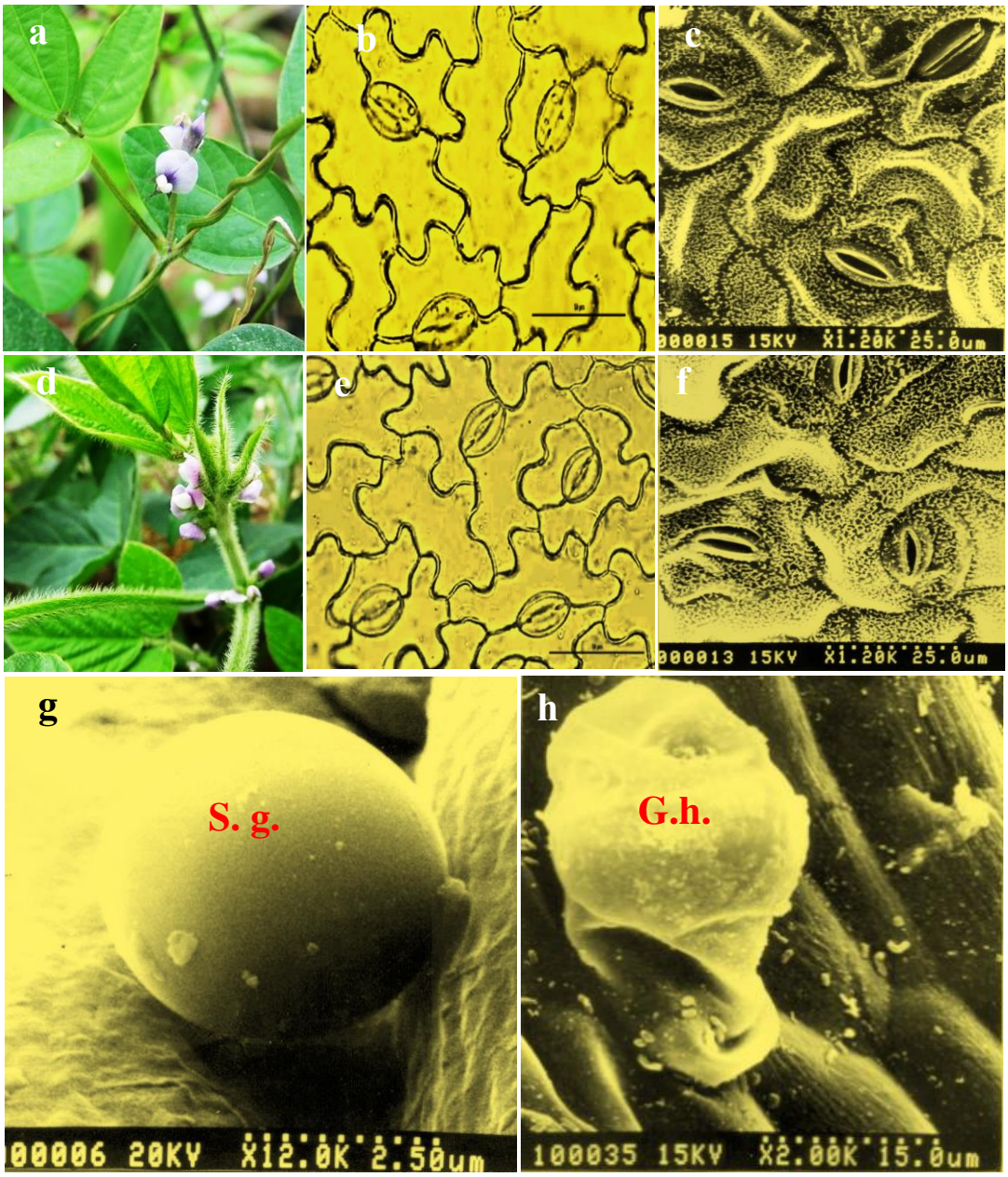
67.Macroscopic and Microscopic Plant Print New Technique for Criminal Investigation of Plants in China, obtain the second prize of technical invention of the *Ministry of Education* in December (2004).

68.Research on the mechanism of plant salt resistance and structural evolution of antagonistic stress in agricultural production, won the second prize of natural science of the Ministry of Education in October (2001).

69.Hegazi, Discovery of Salt glands in Wild Soybean of China" contribution to the study of salt-tolerant plants in the world，Professor Hegazi, a world expert on salt-resistant plants and president of the *Egyptian Desert Research Center*. The reviewer comment (2002).

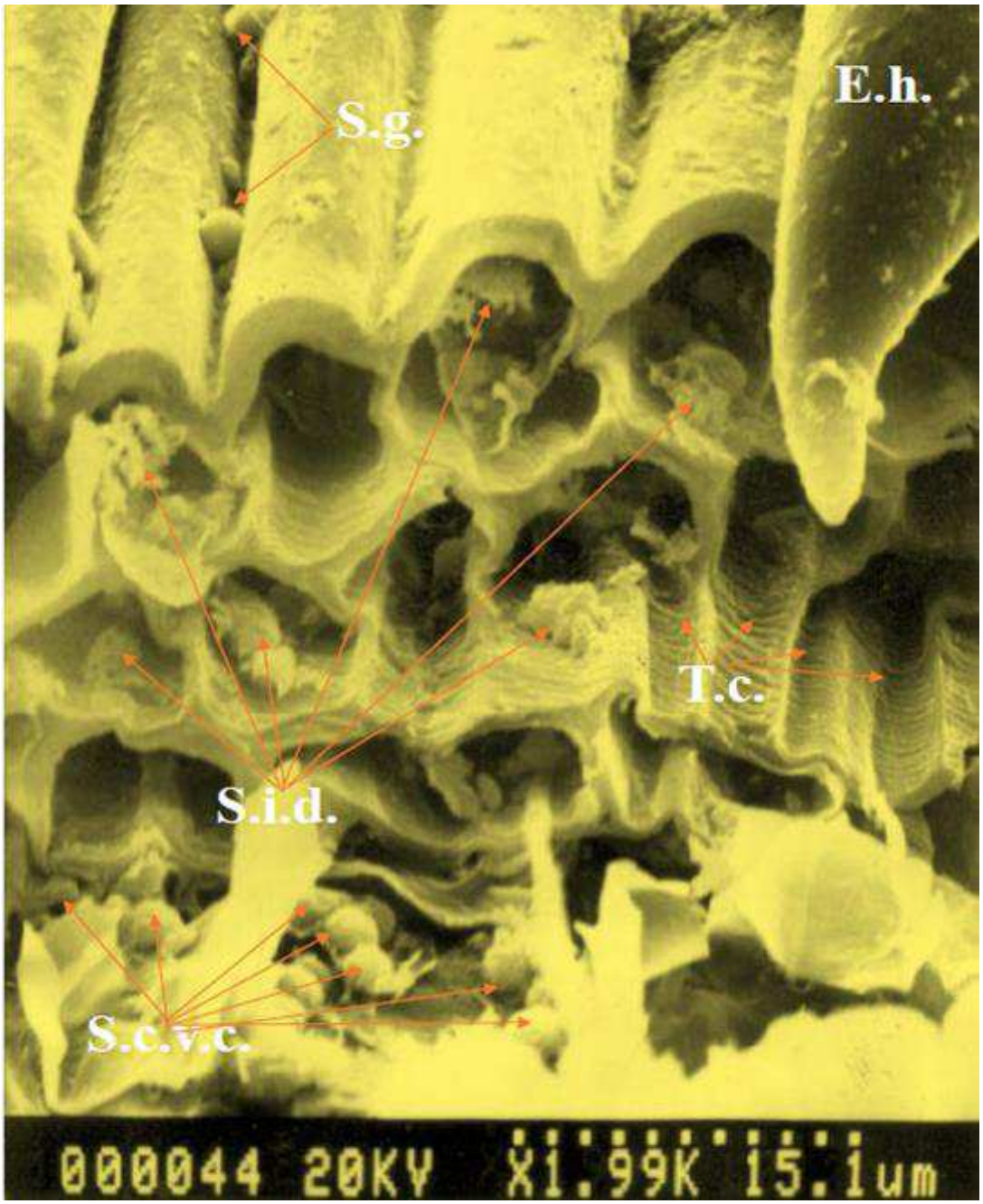


Extended Data Fig. 1 | Plant Print t and Finger Print t are unique and exclusive! Plant Print also has structural stability. a, Finger Print t. **b,** Soybean Plant Print t. Finger Print t and Plant Print t have similar functions, namely: They all have important scientific characteristics that are unique, exclusive and reproducible. **The structure evolution of cotyledon node of wild soybean and cultivated soybean is homologous. c,** Wild soybean with 2 cotyledons and 2 opposite simple true leaves. **d,** Two alternate ternately compound leaf of wild soybean plants. Alternate ternately compound leaf are a hallmark of evolution. **e,** Soybean with 2 cotyledons and 2 opposite simple true leaves. Opposite single leaves are the preservation of the lower morphological and structural evolution of the original type of cultivated soybean development. **f,** Two alternate ternately compound leaf of soybean plants,which have evolved into advanced types. The evolution of cotyledon nodes of soybean and wild soybean has homology



Extended Data Fig. 2 |The results of Plant Print identification of two kinds of soybean have the homology. a, The macroscopic Plant Print of wild soybean, the morphology of stem and leaf**. b,** Leaf apparent structure of wild soybean showed that stomatal guard cells are crescent-shaped, two auxiliary guard cells are parallel and unequal. Radial wall of epidermal cells is inlaid with A type. **c.** Leaf apparent structure of wild soybean Plant Print t: stoma guard cells are crescent-shaped, 2 auxiliary guard cells are in parallel and unequal type, and epidermal cells are inlaid with wall type A. The epidermis has wax coat ornamentation. SEM photograph. **d.** Macroscopic of soybean, shows the stem, leaf and flower. **e,** Leaf apparent structure of soybean Plant Print t: stoma guard cell crescent shape, 2 auxiliary guard cells in parallel and unequal type, epidermal cell wall inlaid A type. **f,** Soybean Plant Print t: stoma guard cell crescent shape, 2 auxiliary guard cells in parallel and unequal type, epidermal cell wall inlaid A type. Wild soybean and soybean have evolved a high degree of homology.

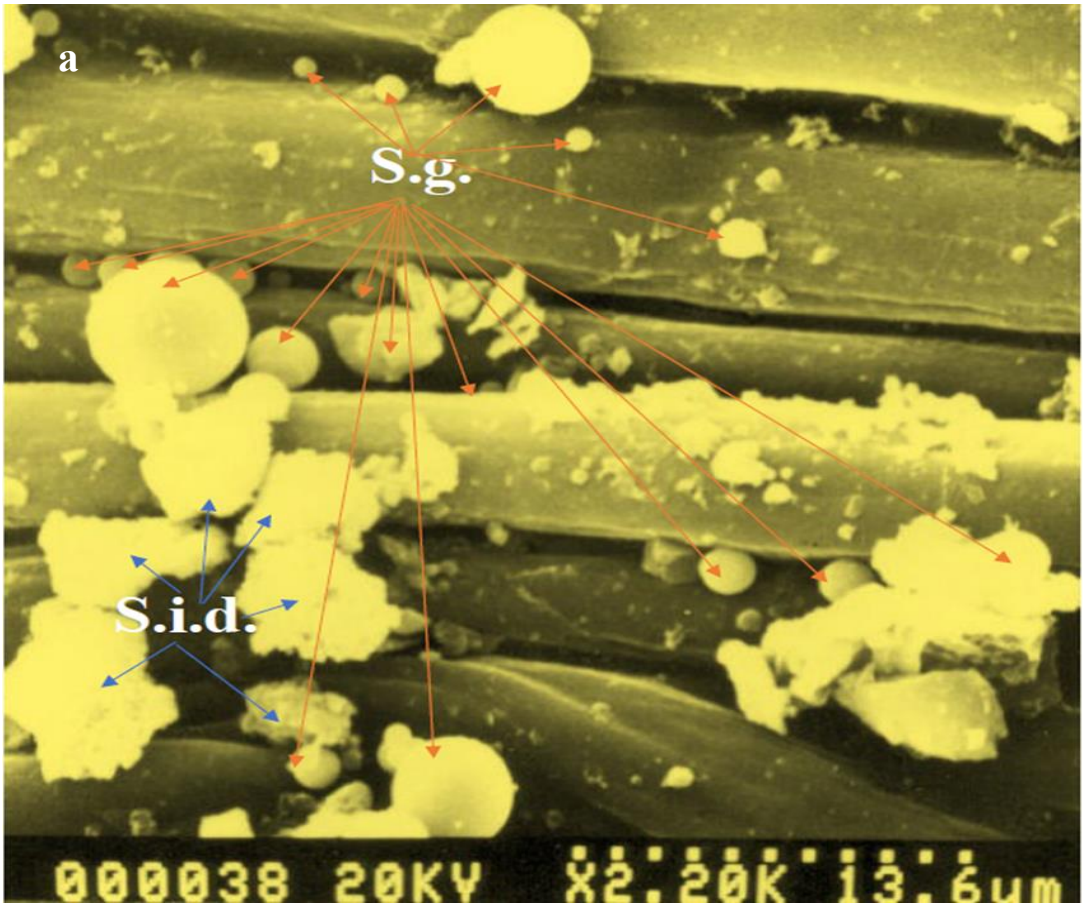
Plant Print distinction between salt glands and glandular hairs of salt-resistant wild soybean by SEM. g, Salt gland of salt-resistant wild soybean. **h,** Glandular hairs of salt-resistant wild soybean. **S. g.** (Salt gland); **G.h.** (Glandular hairs). **SEM (**Scanning electron microscope), **SEM** photograph (**c, e, g, h**), Scale bars, 50μm (**b, e.**).

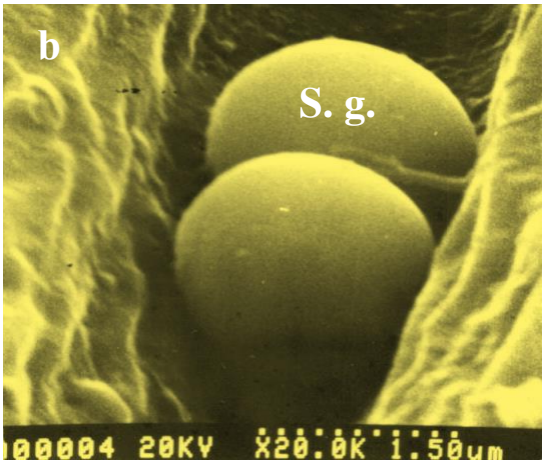


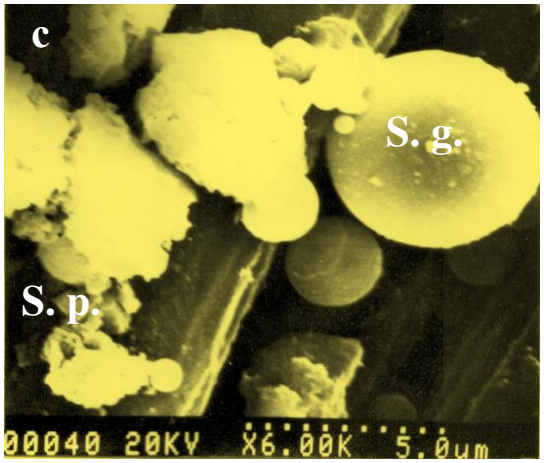
Extended Data Fig. 3 | Cross section of stem of salt-tolerant wild soybean. Showing the epidermal cells of stem, epidermal hair, salt glands, cortical cells, salt-containing vacuole bags. Salt-containing vacuolar capsule can realize the uneven distribution of salt in wild soybean body and reduce the toxicity of Salt to wild soybean. Therefore, salt-containing vacuolar capsule is an evolutionary characteristic rivaling salt stress. Transfer cells can transfer salt-containing vacuoles to salt glands on the surface of wild soybean. Therefore, transfer cells are an evolutionary feature that is resistant to salt stress.

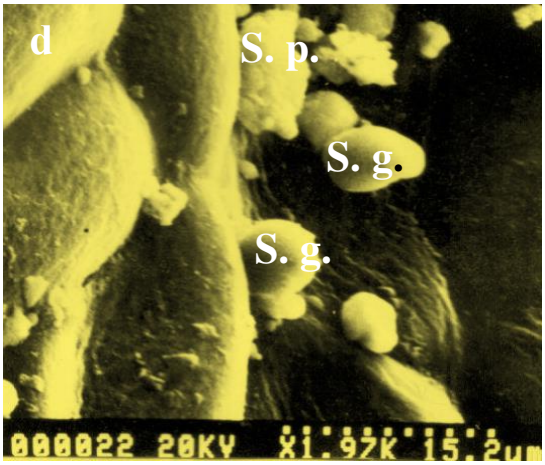
S. g. (Salt glands), **T. c.** (Transfer cells), **S. i. d.** (Salty ions distribution);

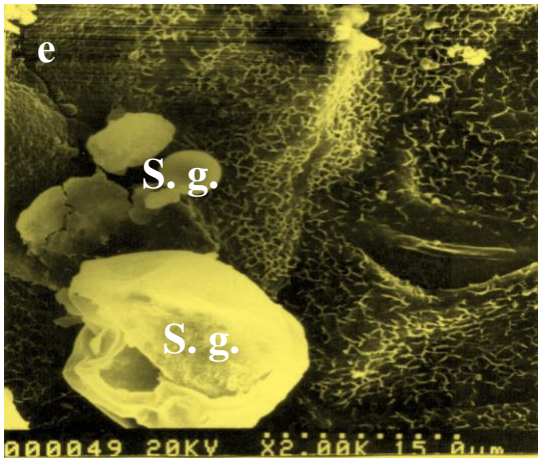
S. c. v. c. (Salt-containing vacuolar capsule), **E. h.** (Epidermal hair). **SEM** photograph.


a
S.g.
S.i.d.
000038 20KV X2,20K 13.6um


b
S. g.
000004 20KV X20.0K 1.50um


c
S. g.
S. p.
00040 20KV X6.00K 5.0um

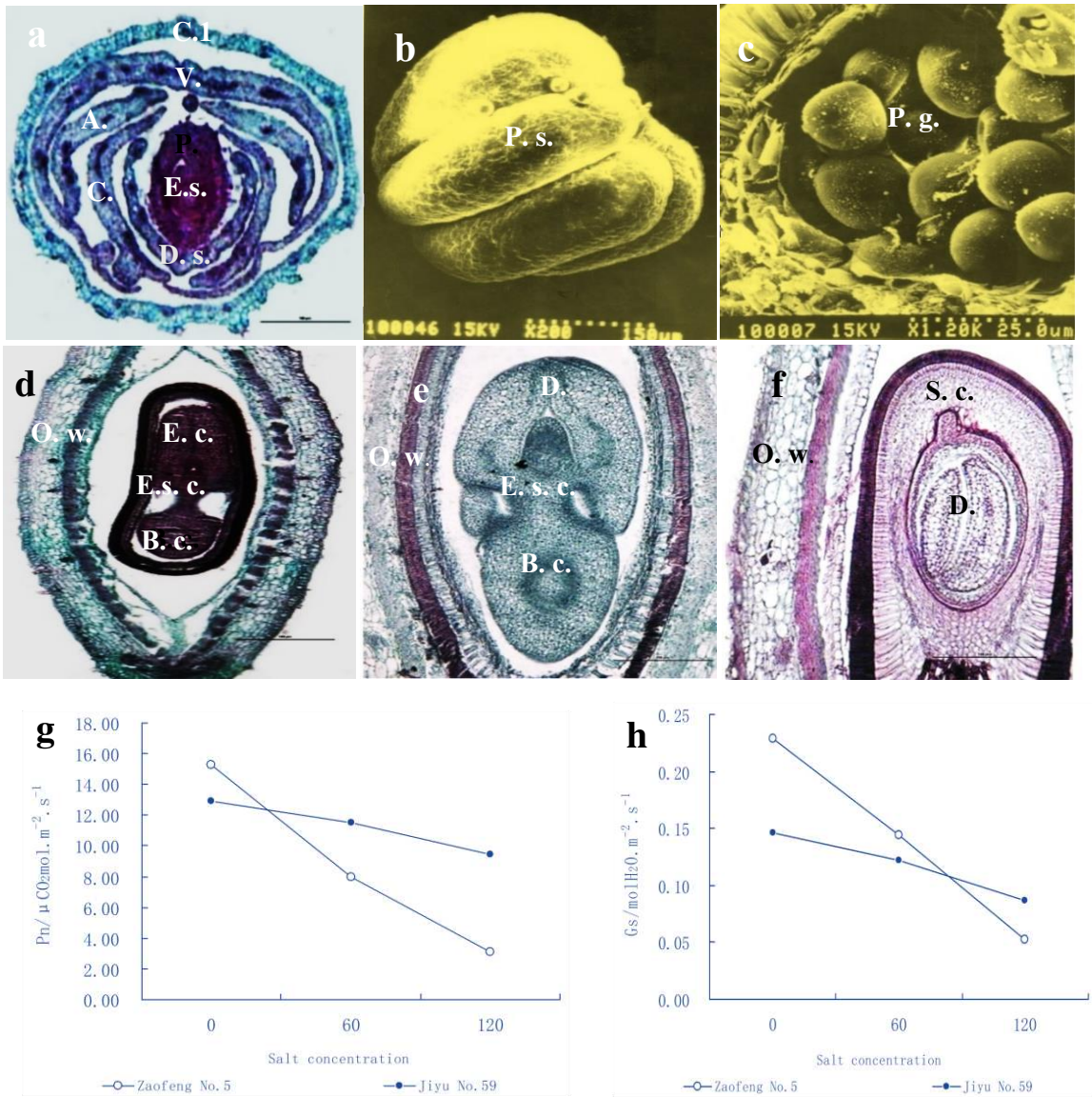

d
S. p.
S. g.
S. g.
000022 20KV X1.97K 15.2um


e
S. g.
S. g.
000049 20KV X2.00K 15.0um

Extended Data Fig. 4 | The apparent structure salt glands of the stem of salt-resistant wild soybean. a, Showing that salt glands are formed layer by layer on the outside of the stem.

Salt glands on the stems and leaves of salt-tolerant wild soybeans in SEM. b, Apparent structure of the stem of saline wild soybean, showing salt gland's head cells and handle cells. **c,** Apparent structure of the stem of salt-resistant wild soybean shows that salt glands fracture release salt particles when they mature. **d,** Apparent structure of the leaf of salt-resistant wild soybean shows that salt glands, i.e. clusters of salt glands. **e,** Leaf apparent structure of salt-resistant wild soybean, show: the wax ornamentations and salt glands attached to the epidermis of salt-resistant wild soybean.

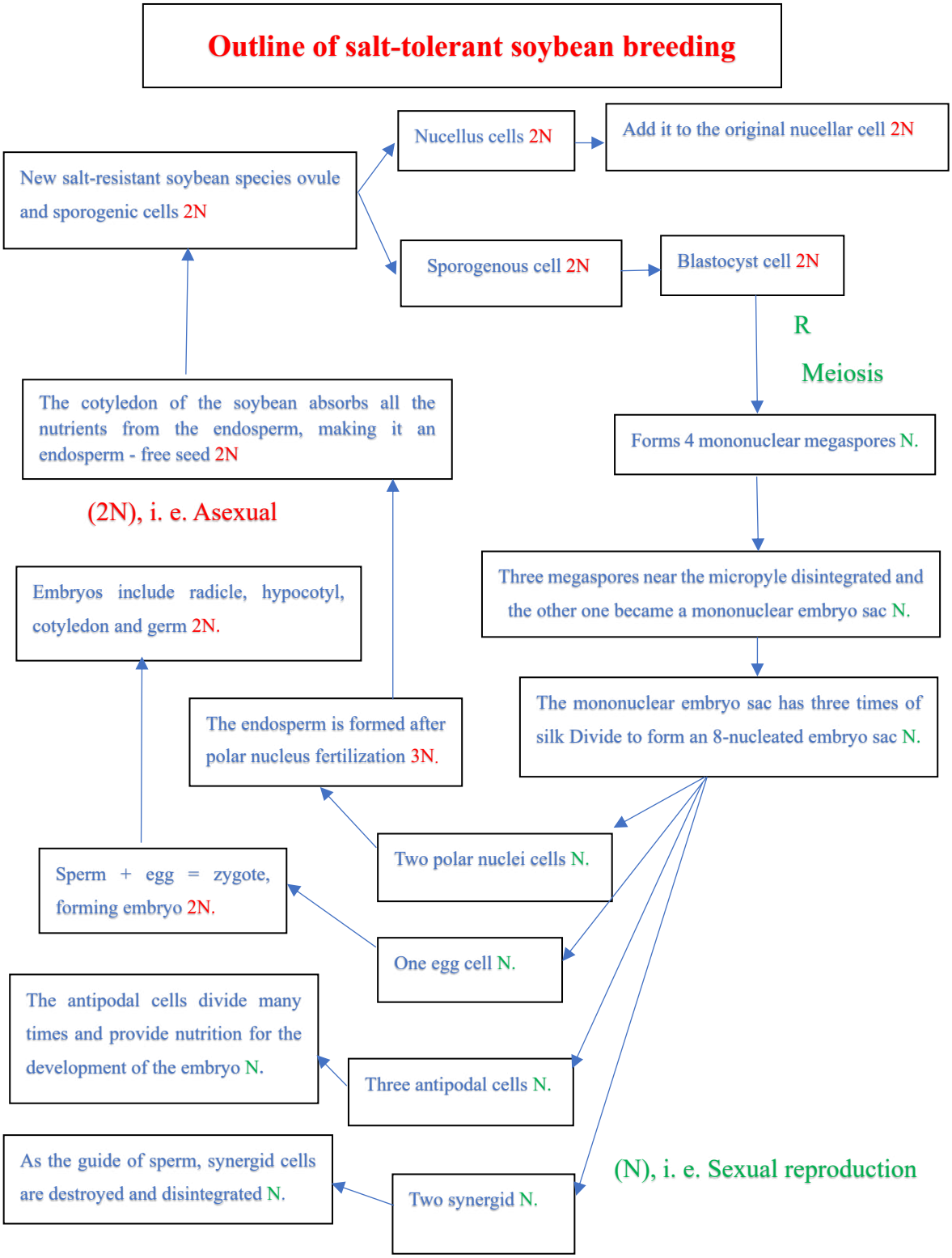
S. g. (Salt gland), **S.p.** (Salt particles), **S.i.d**. (Salty ions distribution). **SEM** photograph. (a, b, c, d, e).



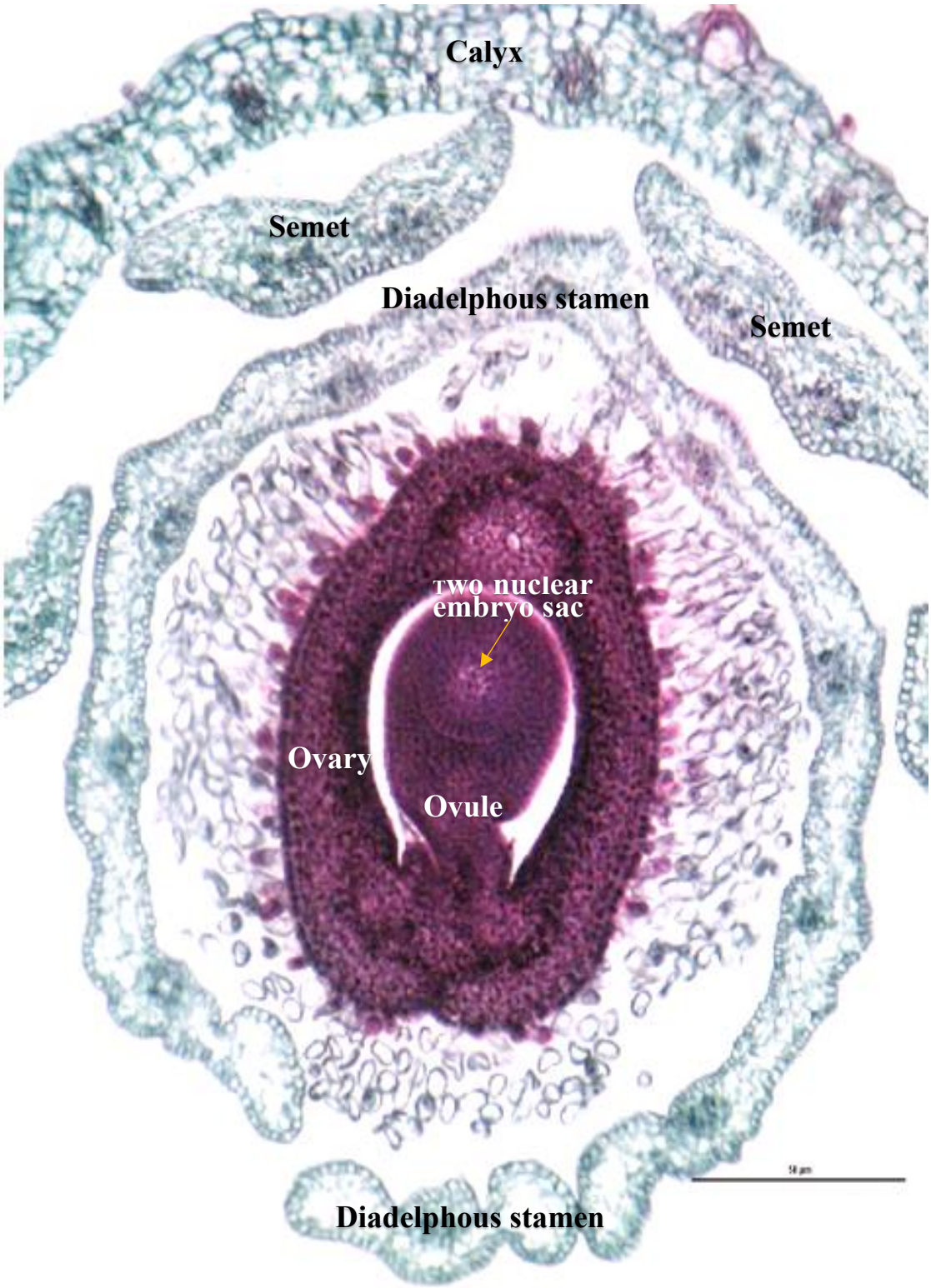
Extended Data Fig. 5 | The salt-resistant soybean breeding. a, Flower structure of salt-tolerant wild soybean structure were transferred to a new salt-tolerant soybean variety. **b,** Pollen sacs of salt-resistant wild soybean. **c,**Using pollen particles of salt-resistant wild soybean to breed. **d,** Successful bred the salt-resistant soybean variety Jiyu No.59. Showing the pericarp and young embryo body of the No.59 cultivated soybean variety, which including: basal cells, embryonic stalk cell and apical embryo cells. **e,** New salt-resistant soybean variety structure, showing the wall of ovary, dicotyledon, embryo, embryonic stalk cell and basal cell of embryo structure. **f,** Ovaries, dicotyledons, embryos, suspensor cells and basal cells of salt-tolerant soybean varieties evolved the structure of salt-tolerant soybean.

g-h, Effects of salt stress on chlorophyll fluorescence in two cultivated soybean leaves were verified. Physiological experiment of stress resistance showed that ability of Jiyu No.59 salt - resistant soybean is very strong.

C.1 (calyx), **V.** (vexil), **A.** (alae.), **C.** (Carina), **P**. (Pistil), **E.s.** (Embryo sac), **D. s.** (Diadelphous stamen (9)+1), **P. s.** (Pollen sac), **P.g.** (Pollen grain), **E. c.** (Embryonic cell), **E.s. c.** (Embryonic stalk cell), **B. c.** (Basal cell), **O. w**. (Ovary wall), **D.** (Dicotyledon), **S. c.** (Seed coat). **SEM** picture (B, c). Scale bars, 100μm (A,D,E,F).

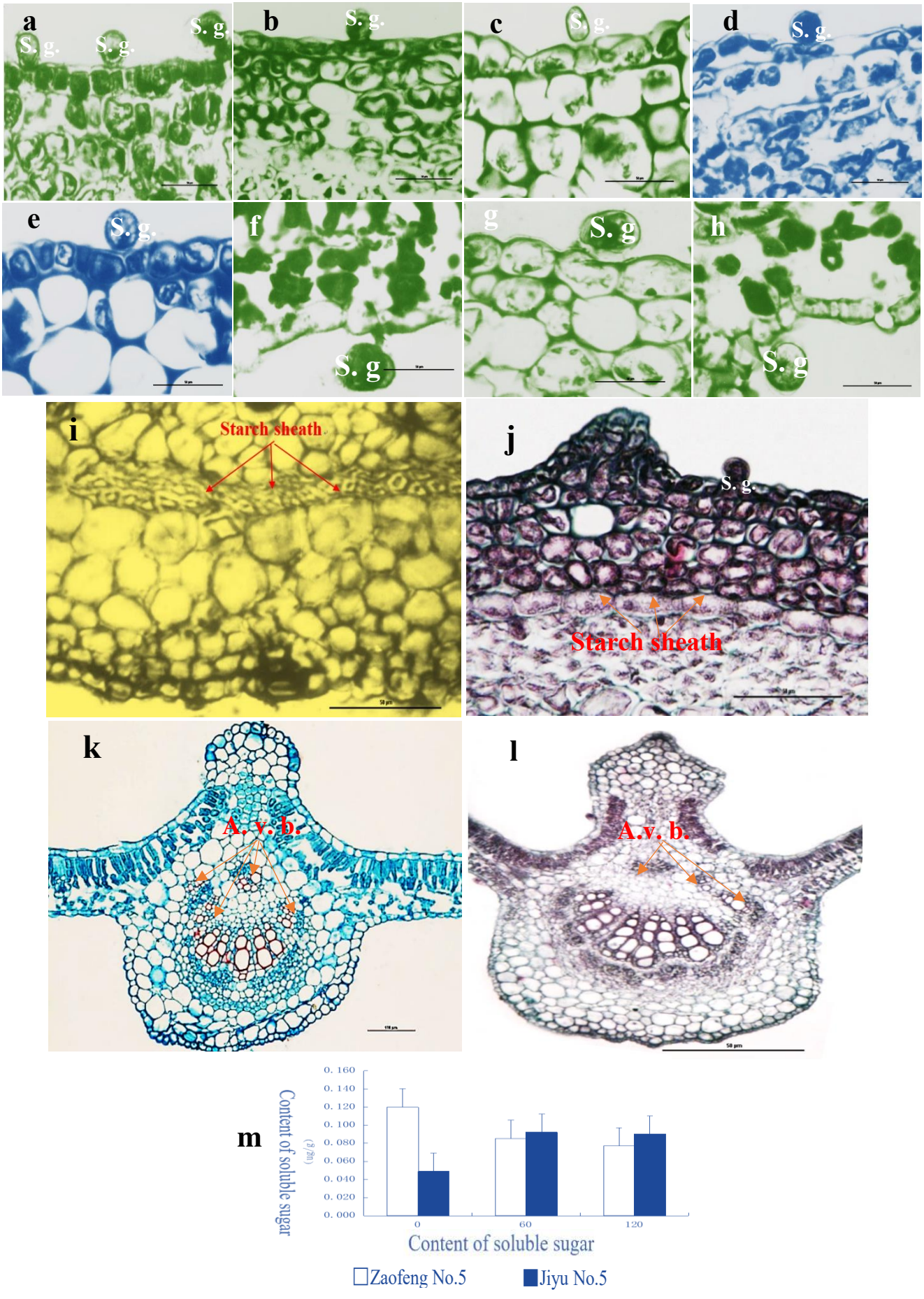


Extended Data Fig. 6 | Schematic diagram of salt-resistant soybean breeding. Ovule and embryo sac development, and embryo formation.



Extended Data Fig.7 | Salt tolerance breeding of soybean, development of ovule and embryo sac.

Showing: Calyx, anther and pollen sac of Salt-resistant soybean plant, filaments of (9) +1 diadelphous stamen, multicellular hairs of outer wall of ovary, development of ovules, ventral sutures bearing erect ovules, vascular bundles of dorsal sutures of ovary wall, 2-nucleated embryo sac. Scale bars, 50μm.


a
S. g.
S. g.
S. g.
b
S. g.
c
S. g.
d
S. g.
e
S. g.
f
S. g
g
S. g
h
S. g
i
Starch sheath
j
S. g.
Starch sheath
k
A. v. b.
l
A.v. b.
m
Content of soluble sugar
0. 160
0. 140
0. 120
0. 100
0. 080
0. 060
0. 040
0. 020
0. 000
0
60
120
Content of soluble sugar
Zaofeng No.5
Jiyu No.5

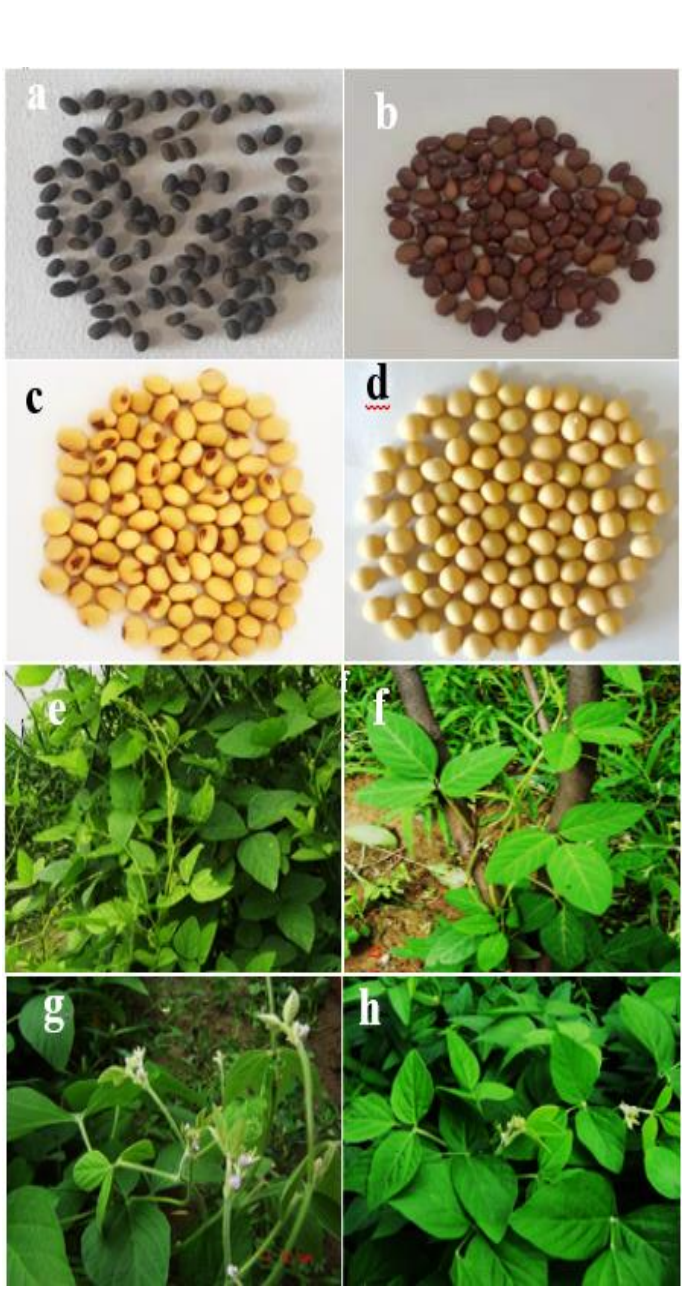
Extended Data Fig. 8 | The salt glands of wild soybean were successfully transferred new soybean varieties. a, II4 stem salt glands and salt-containing vacuoles in epidermal cells. **b, Lf1** showing salt glands and salt-containing vacuoles in cortical and epidermal cells. **c, HG11** showing salt gland. **d, GDPM** stem showing salt-containing vacuoles in salt glands and epidermal cells. **e, g MXD** Salt-containing vacuoles of salt glands, sclerenchyma tissue, and epidermal cells. **f, g MXD** leaves salt glands. **g,** Transverse section of **hL11** showing salt gland and cortical cells. **h, hL11** leaves, showing salt glands at the main veins of leaves.

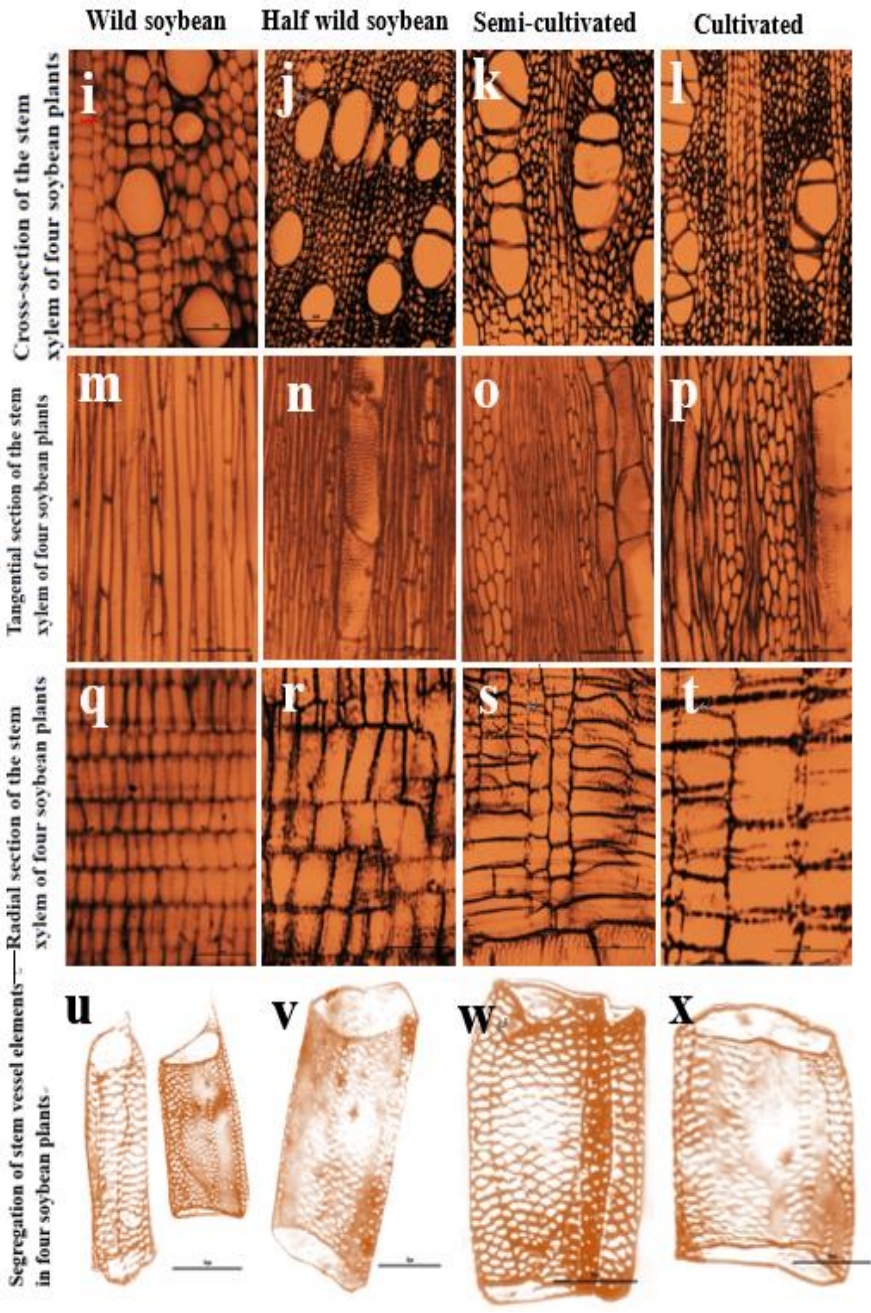
This study is the first discover the endodermal cells of salt-resistant wild soybean stem are filled with starch granules, forming a wall-like starch sheath. i, Stem endodermis cells of salt-resistant No.001wild soybean were filled with starch grains and evolved into starch sheaths. Preventing salt ion from entering the vascular bundles. **j,** Starch sheath of salt-resistant wild soybean was successfully transferred into the cortical cells of cultivated soybean, which effectively improved the salt-resistant ability of JiYu59 salt-resistant soybean.

The salt-resistant wild soybean main veins have the heteromorphism vascular bundles. k, Breeding salt-resistant soybeans, the salt-resistant wild soybean main veins heteromorphism vascular bundles has been transfered to the new salt-resistant JiYu No.59soybean. **l,** Heteromorphic vascular bundle transfusion enhanced the transport function of JiYu59 salt-resistant soybean varieties, and improved the salt-resistant ability of salt-resistant soybean varieties, which is an evolutionary structure feature.

Effects of different concentrations of salt stress on soluble sugar content in Salt-resistant and non-salt-resistant cultivated soybean leaves. **m,** Under salt stress, the sugar concentration of Zao Feng No.5 decreased at 60 mm and 120mM.The soluble sugar content of salt-resistant soybean JiYu No.59 increased with the increase of salt concentration, and the increase was the largest at 120mM concentration, and the content increased by 83.18% compared with the control. The experimental results show that the salt - resistant soybean JiYu No59. Structure evolution has very strong salt - resistance.

S. g. (Salt gland). Scale bars, 50μm (a-j. and k), 100μm (k)


a
b
c
d
e
f
g
h


Wild soybean
Half wild soybean
Semi-cultivated
Cultivated
Cross-section of the stem xylem of four soybean plants
Tangential section of the stem xylem of four soybean plants
Radial section of the stem xylem of four soybean plants
Segregation of stem vessel elements in four soybean plants
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x

Extended Data Fig. 9 | Structural evolution study of four soybean types, include: Seed morphology, color of seed coat, hundred-seed weight and color of umbilicus of four species of soybean. **a,** Seed coat of wild soybean is black, the hilum is black, and the weight of 100 seeds is 1.83g. **b,** Seed coat of semi-wild soybean is maroon red, and the seed hilum is mixed with pink and light white color. Seed weight of 100 seeds is 5.60g. **c,** Semi-cultivated soybean seed coat seeds yellow, hilum brown, 100 seeds weight 12.55g. **d, S**eed coat of cultivated soybean is yellow, the seed hilum is white, and the weight of 100 seeds is 23.35g.

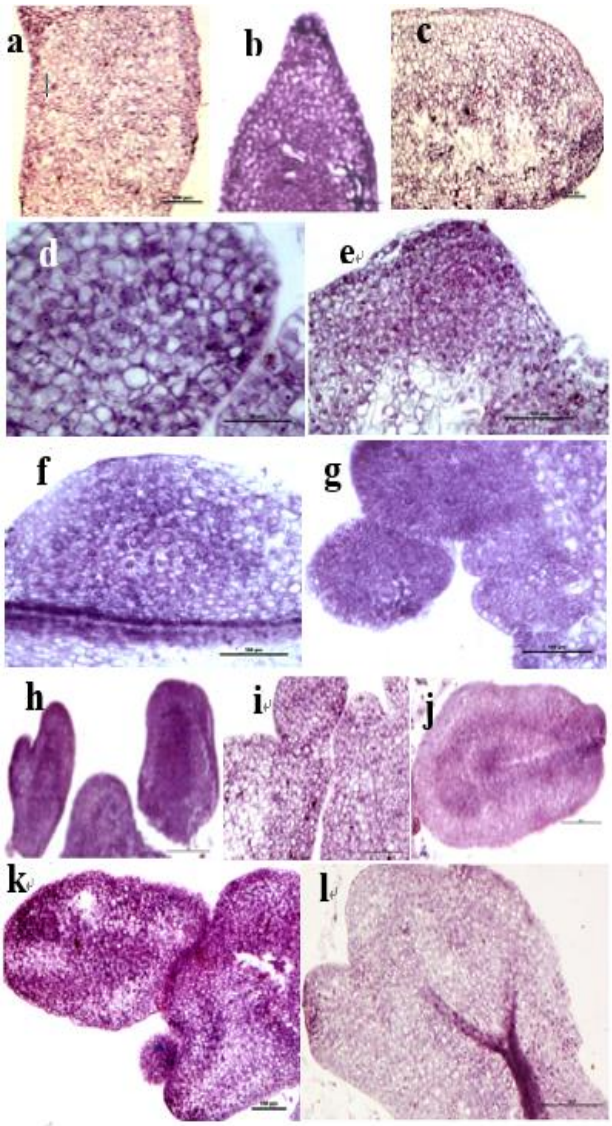
The macroscopic characteristics of four types of soybean are obviously different. e, Wild soybean stems are thin, straggly, without epidermal hair, and the differentiation between main stem and branches is not obvious. **f,** Semi-wild soybean, twining stem, with epidermal hair. **g,** Semi-cultivated soybean when the stem grows to 28 ~ 30cm high, the main stem rapidly evolves into straggly stem. **h,** Cultivated soybean main stem is strong and distinct from branches. The experimental results provide important classification evidence for salt-resistant soybean breeding.

The structural evolution identification of four soybean types was carried out to screen the fathers of salt-resistant wild soybean. i, Cross section of the stem of salt-resistant wild soybean plant shows that vessel elements are single tube hole. **j,** Cross section of the stem of the semi-wild soybean plant vessel elements with 2 cells of compound tube hole chain, which are connected by radial tube hole chain **k,** Cross section of the stem of semi-cultivated soybean plant shows that vessel elements are mostly complex tube hole chain. **l,** Cross section of the stem of cultivated soybean plant shows that vessel elements have radial tube hole chain connections. **m,** Tangential section of the stem xylem of a wild soybean plant, showing a single row of rays. **n,** Tangential section of the stem xylem of a semi-wild soybean plant, showing 2 rows of rays. **o,** Tangential section of the stem xylem of a semi-cultivated soybean plant, showing multiple rows of rays. **p,** Tangential section of the stem xylem of a cultivated soybean plant shows many rows and intensive rays. **q,** Radial section of the stem of a wild soybean plant showing an erect vascular ray shape. **r,** Radial section of the stem material of hemi wild soybean, showing the vertical and recumbent vascular rays. **s,** Radial section of the stem of a semi-cultivated soybean plant shows that the vascular rays are less upright and more recumbent. **t,** Radial section of the stem of a cultivated soybean plant shows a recumbent vascular ray shape.

The four types soybean structural evolution identification of segregation of the stem xylem. u, Showing the end wall and interrow pore pattern of vessel elements. **v,** Segregation of the stem xylem of a hemi wild soybean plant, showing end walls and lateral walls of vessel elements and interrow pores. **w,** Segregation of the stem xylem of a semi-cultivated soybean plant, showing end walls and interrow pores of vessel elements. **x,** Segregation of the stem xylem of cultivated soybean plant, showing: end-wall and interrow pore type of vessel elements.

The results of three sections and segregation experiments of four different types of soybean showed that wild soybean was the original position in the system evolution, while cultivated soybean was the advanced position in the system evolution

Scale bars, 50μm. (i-l and q-x). Scale bars, 100 μm. (m - p).



No.07.Non-salt-tolerant soybean has died in the soil at pH8.9.

No.59. Salt-tolerant soybeans thrived on the same ridges in pH8.9 soil.

Extended Data Fig. 10 | Embryogenesis in cross section of tissue culture explants of salt-tolerant new soybean varieties. a, Cross section of the explants after 5 days of inoculation showed that germinating cells began to appear, with deep staining, **b,** Transverse section of explants after 9 days of inoculation and culture, showing that the internal tissue had formed pseudo-callus, **c,** The cross section of the explants after 11 days of culture showed that the nuclei of the explants were significantly enlarged and stained, and the state of the cells before division, **d,** The transverse section of the explants after 14 days of inoculation showed that the cells in the epidermal cells divided vertically and peripherally to form four cell embryos. **e,** The transverse section of the explants after 18 days of inoculation showed that the internal nuclei were enlarged and began to germinate. **f,** The transverse section of explants inoculated and cultured for 20 days showed that embryo bodies were basically formed. **g,** The explants were inoculated for 20 days, indicating that the length of embryo body was closely related to the explants. **h,** The transverse section of explants inoculated for 25 days showed the formation of anisomorphic embryos and spherical embryos in dicotyledon. **i,** The cross section of the explants after 25 days of culture showed that several spherical embryos developed into soybean embryos. **j,** The transverse section of the explants inoculated and cultured for 30 days showed that the embryo body completely formed protruding explants surface, and formed completely independent yu Lei embryo**. k,** Transverse section of explants inoculated for 30 days showed a bud beside the heart-shaped embryo. **l,** Transverse section of explants inoculated and cultured for 30 days showed that vascular tissue was independent from the center of the embryo.

Study shows that the new salt-resistant soybean species had strong resistance to salt-soaked environment.m, The common cultivated soybeans on the pH8.9 same ridge that are not salt-resistant gradually wither and die because of salt stress. **n,** However, the new salt-resistant soybean "Jiyu 59" thrived by using the wild soybean with salt gland as the male parent breeding of new varieties, which is grow luxuriantly. The two soybean varieties plants were a clear contrast. The important role of the structure evolution of salt - resistant soybean under salt - alkali stress and the identification of Plant Print were verified. Therefore, this study has effectively solved the problems of saline-alkali soil improvement, development and utilization under certain conditions. **o,** The new salt-resistant soybean "Jiyu 59" fruits. **p,** Show: Pod and seed of salt-resistant soybean "Jiyu 59". **q,** Comparison of seed size and morphology between No.07 no salt-resistant soybean and No.59 salt-resistant soybean. **r,** The results showed that the salt-resistant soybean"Jiyu 59" had a good harvest in the salt-soaked environment.

Scale, 100μm (a, b, c, f, g, j, k, l), Scale, 50μm (d, e, h, i).