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Chapter from the book *A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships*

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1. Introduction

Agriculture requires ~70% of the total fresh water resources [1]. Agricultural drought refers to the shortage of precipitation that causes deficit in soil water and reduction of ground water or reservoir levels, which will hamper farming and crop production [2]. To assess the severity of agricultural drought, different indices based on various parameters have been adopted. An expert meeting of the World Meteorological Organization was held in 2010 in Geneva to discuss and categorize the recently in-use indices into 7 types (Table. 1) [3]. With the establishment of networks under the cooperation of authorities at different administrative levels, one purpose is to develop monitoring tools and early warning systems for droughts. Nevertheless, a universal agricultural index satisfying all common interests has not yet arrived.

The effects of drought on soybean have been extensively reported, including morphological changes of the vegetative plant and the reduction in seed quantity and quality. Methods for assessing both quantitative and qualitative morphological parameters have been reported. Tremendous efforts have been placed on the enhancement of drought tolerance of soybean, with a primary goal of enhancing yield under drought. Traditional breeding is a widely accepted strategy which will combine desirable agronomic traits from soybean germplasms, via repeated crossing and selection processes. The recent advances in genomics, genetics, and molecular biology facilitate the identification of molecular markers and functional genes that are related to drought tolerance in soybean. Therefore, the ideas of enhancing drought tolerance by marker-assisted breeding and genetic modification have gained growing attention.
### Table 1. Types of commonly used drought indices.

| Types of indices                                    | Examples                                                                 | Advantages                                                                 |
|-----------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Precipitation-based                                 | Standardized Precipitation Index; Days without Rainfall                  | Simple; required data can be easily accessed                              |
| Temperature-based                                   | Cold Spell Duration Index; Warm Spell Duration Index                      | Straightforward monitoring of trends in the frequency or intensity of events |
| Precipitation- and temperature-based                | Palmer Drought Severity Index; Standardized Precipitation Evapotranspiration Index | Simple to apply; more comprehensive                                        |
| Precipitation, temperature, and soil moisture/ soil characteristics based | Relative Soil Moisture; Accumulated Drought Index | Better understanding about the interaction between crops and the environment during drought; help determine the influence of drought on the crop growth and yield |
| Temperature, relative humidity, solar radiation, wind speed, and soil moisture/ soil characteristics based | Aridity Anomaly Index; Soil Moisture Anomaly and Relative Soil Moisture Index | Fully consider both crop and soil water supply and demand |
| Remote sensing-based                                | Normalized Difference Vegetation Index; Temperature Condition Index      | Required spectral data from instruments are easily available for most parts of the world; can monitor vegetation conditions |
| Composite                                           | Utilize and incorporate a consolidation of indices into a comprehensive one | Comprehensive and flexible for monitoring different types of drought in different places |

In addition to the genetic improvement programs for soybean, agronomic practices aimed at minimizing water input, reducing water loss, and increasing plant water usage efficiency have also been developed to cope with the problem of water scarcity. Some of these can be applied for soybean cultivation.

In this chapter, we will summarize the understandings of drought stress and drought tolerance in soybean from available literatures. We have integrated information from traditional breeding and agronomic measures to molecular aspects of this subject, and highlighted unsolved problems and possible strategies to tackle them.

## 2. Effects of drought on soybean production

Soybean is among the top 10 of the most widely grown crops, with a total production of over 260 million tonnes in 2010 (FAO data). The cultivated area of soybean occupies more than 100 million hectares worldwide, with about half in the U.S.A. and Brazil (FAO data). Greenhouse and field studies showed that drought stress led to significant reduction in seed yield (24–50%) from distinct locations and time [4, 5].
Numerous efforts have been put to examine the effects of drought and irrigation at various vegetative stages on soybean production. A 2-year field experiment by Brown et al (1985) on 4 determinate cultivars Davis, Lee 74, Sohoma and Centennial demonstrated that moisture stress initiated at R2 or R4 reduced yield significantly [6].

An in-depth analysis of the effects of drought at various growth stages on seed yield of soybean cultivar Douglas was reported by Eck et al (1987) [7]. In their study, yield loss was the most severe when drought stress was applied throughout the seed development period (R5-R7), resulting in a reduction of 45% and 88% respectively in two consecutive years [7]. Besides, Desclaux et al (2000) conducted a comprehensive analysis of yield components when drought stress was applied to soybean cultivar Weber at different developmental stages [8]. In this experiment, the stress condition was attained by temporally withholding irrigation for 4 to 5 days until the plant available water reduced to 50% or 30% of the normal conditions. The major findings showing various adverse effects of drought were summarized in Table 2. The most severe effect of this treatment was observed during the seed filling period [8].

On the other hand, Korte et al (1983) conducted a 3-year study on 8 soybean cultivars to assess yield enhancement by irrigation, using non-irrigated soybean plants as the control group [9]. The experimental groups were irrigated at different developmental stages (one stage or different stages in combination), including the flowering stage (R1-R2), the pod elongation stage (R3-R5), and the seed enlargement stage (R5-R6) [9]. Results of factorial analysis indicated that the yield was sensitive to the enhancement by irrigation, at pod elongation stage (R3-R4) and the seed enlargement stage (R5-R6) [9]. For 5 cultivars, the enhancement effect by irrigation followed the order: seed enlargement stage (R5-R6) > pod elongation stage (R3-R4) > flowering (R1-R2) [9]. A separate experiment by Kadhem et al (1985) supported the sensitivity toward irrigation at the pod elongation stage in determinate cultivars (R3.7 and R4.7) [10].

| Traits | Growth stage with drought stress application |
|--------|--------------------------------------------|
|        | Vegetative | During | Pod | Seed Filling |
|        |           | Flowering | Lengthening | |
| Dry matter value | Main-stem height | * | * | |
|          | Internode length | | | |
|          | Number of nodes | * | | |
|          | Stem diameter | | | |
|          | Leaf surface area | * | | |
|          | Pods per dry matter | | | *
| Yield components | Seeds per pod | * | | |
|          | Individual seed weight | * | | *

* indicates significant effect of drought on the trait. Growth stages were characterized by the Fehr and Caviness scale [11]. Experiments were carried on the indeterminate cultivar, Weber.

Table 2. Effects of drought at different developmental stages on different agronomic traits (Modified from [8])
It has been clearly demonstrated that water availability will affect seed yield, though the growth stages that are most sensitive to drought stress vary among reports on different cultivars. In contrast, there are controversial reports on the effects of drought on soybean seed quality. Germination rate is a crucial criterion for assessing seed quality. A 2-year field study conducted on 3 soybean cultivars of Maturity Group (MG) IV, V and VI respectively in the southern U.S.A. reported a reduction of seed germination to less than 80% of the control, when drought stress was applied at any of the tested reproductive stages [12]. This observation is supported by a greenhouse experiment reporting that the germination rate was reduced in medium seeds from plants subjected to drought during seed filling period [13].

On the contrary, in a greenhouse experiment using the cultivar Gnome [14], drought stress led to a reduction of seed yield mainly due to the reduction of seed number. Nevertheless, there were only slight reductions in standard germination percentage and seedling axis dry weight of the harvested seeds. The authors suggested that drought stress affects the seed yield to a larger extend than seed quality. This result is supported by a separate experiment using other determinate and indeterminate cultivars (Essex, Union, Harper and McCall), in which drought did not result in production of seeds with reduced germination rate or vigor, except for those shrunched, flat, and underdeveloped seeds [15].

The study by Dornbos and Mullen (1991) further showed that the effect of drought on the germination rate of seeds from stressed plants was more significant when the air temperature reached 35°C. The authors also reported an increase in the percentage of hard seeds with increased duration of drought stress, and a negative relationship between seed weight and the percentage of hard seeds [16]. Hard seeds possess impermeable seed coats that will impede germination. In conclusion, drought clearly affects seed quality on some soybean cultivars. However, the discrepancies among different reports suggest that such effects are not universal to all cultivars under different stress conditions.

The contents of seed protein and oil are major parameters determining the nutritional value of soybean. Soybean seed protein content in general is negatively correlated with the amount of seed oil [17]. A differential irrigation experiment performed on soybean cultivars Gnome and Hodgson 78 in a greenhouse setting reported a 4.4% increase in protein content and 2.6% decrease in oil content under severe drought [18]. Furthermore, a 6-year field experiment was conducted using 60 soybean cultivars and breeding lines (Figure 1). The results confirmed both the negative correlation between seed protein and seed oil contents as well as the effect of drought on seed protein and seed oil contents [19]. The variations in contents of seed protein and oil were attributed largely to the differential rainfall during the seed filling stage [19].

Soybean seeds are also rich in isoflavones, a group of secondary metabolites exhibiting estrogenic, antifungal, and antibacterial activities [20]. The level of isoflavones is affected by drought during seed development [21]. While drought stress reduced the total content of isoflavones in soybean seeds under 28°C and 700 ppm CO₂, an increase was observed when the drought stress was applied at 23°C and 700 ppm CO₂ [21]. The results implied that the isoflavone content in soybean seeds is responsive to drought but also to other environmental factors including temperature and CO₂ level.
3. Parameters for measuring the degree of drought stress in soybean

3.1. Parameters related to seed

Seed weight can be evaluated using 100-seed weight or seed weight distribution. To eliminate the effects of the large measurement errors on the weight of a single seed, the weights of batches of 100 seeds are measured instead. Despite the general decreasing trend of seed weight under drought, the seed weight may not reduce uniformly as a function of drought intensity [16]. Therefore, seed weight distribution has become another parameter employed to evaluate the effect of drought on seed weight, through the assessment of weight of seeds of different sizes. Dornbos and Mullen (1991) reported that under severe drought, the proportion of seeds of diameter larger than 4.8mm was reduced by 30%-40% while the proportion of seeds of diameter smaller than 3.2mm was increased by 3%-15%. Under drought, soybean plants continued to produce heavy seeds. However, a greater portion of seeds were of low weight [16].

3.2. Parameters related to vegetative tissues

Drought stress reduced the number of nodes which is a result due to the reduction of main stem height and the decreased node emergence rate [8]. Length of internode is also a parameter for evaluating drought stress. However, the change in internode length is dependent on the timing of drought. In the experiment reported by Desclaux et al (2000), only the internodes which initiated during drought stress showed reduction in length [8].
Reduction in leaf area is a convenient morphological parameter for measuring drought stress experienced by the plant. Commercially available leaf area meters provide a non-destructive means to measure leaf area in the field. Alternately, the area of detached leaves can be measured simply by creating a digital image of the leaf using desk-top scanners followed by image analysis by computer software [22].

Drought stress also leads to a reduction in leaf relative growth rate [23], which can be calculated using the following formula:

\[ \text{RGR} = \frac{\ln(\text{FDW}) - \ln(\text{IDW})}{(t_2 - t_1)} \]

where, FDW refers to the final dry weight; IDW refers to the initial dry weight; \( t_2 \) refers to the time in days at the end of the experiment; \( t_1 \) refers to the time in days at the beginning of the experiment.

The degree of chlorophyll reduction in soybean leaves was correlated with the strength of drought treatments [24]. Chlorophyll can be simply extracted by immersing the plant tissue in N,N-dimethylformamide (DMF) [25]. After incubation and mixing, the DMF is subjected to OD determination. The total chlorophyll content is calculated as 

\[ C_t = 8.24 A_{664} + 23.97 A_{647} - 16.64 A_{603} \]

where \( C_t \) is the total chlorophyll content in \( \mu g/ml \) of the DMF subjected to measurement [25].

4. Accessing drought tolerance of soybean

To facilitate cultivation of soybean in semi-arid and arid regions, it is important to rank soybean cultivars according to their drought tolerance. Various parameters have been adopted to assess drought tolerance (Table 3). Due to different environmental and temporal factors, the results of assessment may be varied. Therefore, experiments have to be conducted consecutively for a few years in the same regions with large sampling size using various assessment parameters in order to achieve a more reliable classification.

| Index | Description | Refs |
|-------|-------------|------|
| A. Direct yield scoring methods | | |
| 1. Average yield | Average yield of certain germplasm in arid region or in the same region for several years. | [26] |
| 2. Water usage efficiency | Unit yield on the expense of a unit of water. | [26] |
| 3. Environmental index | Yield at targeted region relative to the average yield of all regions tested. | [26] |
| B. Drought tolerance coefficient-based methods | | |
| 1. Drought tolerance coefficient (I) | Yield at water deficit year/region relative to water sufficient year/region. | [26] |
| Index | Description | Refs |
|-------|-------------|------|
| 2. Drought tolerance index (DI) | DI = (I x YD)/AVER<sub>yield</sub> where I is the drought tolerance coefficient, YD is the yield of targeted germplasm in arid region, AVER<sub>yield</sub> is the average yield of all germplasms tested in the same field. This method accounts for the fact that a germplasm that has a higher drought tolerance coefficient can actually have a lower yield. | [26] |

C. Others

1. Germination stress index (GSI) | Soybean seeds are germinated in water or hypertonic solution. GSI = PI<sub>treat</sub>/PI<sub>control</sub> where PI is the summation of the germination rate at day 2, 4, 6, 8 in a ratio of 1, 0.75, 0.5 and 0.25 respectively. | [27] |

2. Survival rate | Water is withdrawn during the 3rd – 5th trifoliate stage. Time needed for 50% of plant to die is determined. | [26] |

3. Repeated drought during seedling stage | Water is withdrawn from seedlings at 1st trifoliate stage. The seedlings are re-watered when half of the seedlings are suffering from permanent wilting. This process is repeated 2-3 times to determine the amount of seedlings that can recover from the treatment. Switching between hypertonic solution and water can also be used as treatment. | [26] |

4. Membership function method | To compare performance of different germplasms under the same standard, each agronomic trait of each germplasm was converted to membership function using the principle of fuzzy logic. Germplasms can be ranked using the membership function into 5 levels at which 1 is the most tolerant and 5 is the most sensitive. | [28] |

5. Canopy wilting index | Degree of wilting of the canopy is determined during the mid-day of fine weather. Degree of wilting can be ranked into 6 levels from 0 to 5, where 0 and 5 represent no wilting and completely wilted, respectively. | [26] |

D. Methods used officially in China

1. Tolerance during germination | Seeds are air-dried at 20°C before experiment. 100 seeds are germinated either in water-soaked (control) or 40% PEG-soaked (treatment) cotton at 25°C. Experiment is conducted with triplication. Germination rates are determined 7 days later. Relative germination rate (RGI) is calculated by dividing the treatment germination rate with control germination rate. Level 1: RGI >95% Level 2: RGI between 80% and 95% Level 3: RGI between 65% and 80% Level 4: RGI between 35% and 65% Level 5: RGI < 35% | [29] |
Test should be carried out in field with precipitation less than 50mm. Seeds of each germplasm in each treatment are sown in single row of 1.5 m. The control field is irrigated (7 times) to maintain the field soil moisture. In the treatment field, irrigation is only applied before sowing to ensure the germination of seeds. Plant height, number of branching, number of pods per plant and yield per plant of 10 plants are determined upon harvest. Drought tolerance coefficients of each trait are calculated. Average of coefficients (RI) of all traits will be used to rank the germplasm into 5 levels.

- Level 1: RI > 0.6500
- Level 2: RI between 0.5000-0.6500
- Level 3: RI between 0.3500-0.5000
- Level 4: RI <0.3500
- Level 5: Plant died or cannot reproduce

Table 3. Common parameters for assessing drought tolerance of soybean cultivars.

5. Morphological and physiological adjustments of soybean under drought stress

For ease of discussion, we define the term drought tolerance loosely to include all mechanisms that allow soybean to survive better under drought. Soybean cultivars of different drought tolerance exhibit a spectrum of differential morphological and physiological changes under drought stress, presumably due to the differences in their genotypes.

5.1. Morphological and growth adjustments

Morphological adjustments are sometimes effective means to avoid drought stress. A number of root-related traits have been proposed as indicators of drought tolerance in soybean [30-34]. Root distribution, which is measured in terms of horizontal and vertical root length density or dry matter in soil of different depth [34, 35], will change in drought tolerant soybean cultivars under drought stress [36]. It was reported that under seasonal drought, there is a low root density in the dry surface soil but a high root density in the deeper region of the soil where the water content is higher [34]. Moreover, using data from drought tolerant soybean cultivars, it was found that there is a positive correlation between drought tolerance and dry root weight/plant weight; total root length/plant weight, and root volume/plant weight [30].

Root to shoot ratio increases under water deficit conditions [37]. It has been proposed that the cessation of shoot but not root growth can be explained by the higher sensitivity to water deficit of shoot than root [37]. The differential growth is closely related to the differential
change in cell wall composition, which involves the thickening of shoot cell wall and relaxing of the expansion of root cell wall by certain catalytic enzymes and stiffening agents [37]. There are only limited reports on related studies in soybean. The study on GmRD22 from soybean suggested a relationship between osmotic stress and cell wall metabolism. GmRD22 is a BURP-domain containing protein localized in the apoplast, which may play a role in stress tolerance by regulating lignin content of cell wall under stress, presumably through interacting with cell wall peroxidases [38].

The adjustments of leaf morphology may play a role in drought tolerance. Some cultivars take advantage from the maintenance of leaf area which provides a possible benefit for the growth of soybean plant after the stress is relieved [39]. Under stress, drought tolerant soybean cultivars exhibited a larger leaf area when compared with less tolerant cultivars [23, 35]. This phenomenon was associated with the larger extent of reduction in stomatal conductance and yet a smaller extent of reduction in photosynthetic rate in the tolerant cultivar [23]. In this case, the drought tolerant cultivar may benefit from the reduction of water loss while minimizing the cost of reduction of photosynthesis.

5.2. Physiological and biochemical adjustments

To survive over an extended drought period, it is important for the soybean leaves to adjust its stomatal conductance to prevent excessive water loss. For example, after 30 days of water stress, the drought tolerant soybean variety MG/BR46 exhibited a higher degree of reduction in stomatal conductance when compared to the drought sensitive cultivar BR16 (65% versus 50% reduction) [23]. After 45 days of stress, the reduction in stomatal conductance was no longer detectable in the sensitive cultivar while it had reached 79% in the tolerant cultivar [23].

Another important adjustment under drought stress is to maintain cell turgidity. In a field test conducted using the drought tolerance soybean cultivar PI 416937 and the sensitive cultivar Forrest, it was found that PI 416937 maintained a lower solute potential yet a higher water potential and water use efficiency. As a result, PI 416937 gave a higher seed weight and yield than Forrest under drought. This report provided evidence on the positive correlation between turgor maintenance of leaves and drought tolerance [40].

To maintain cell turgidity under stress, osmotic adjustment is a common mechanism which involves active accumulation of solutes in cells [39]. In soybean, drought stress up-regulates the expression of the soybean P5CS gene which encodes the enzyme Δ1-pyrroline-5-carboxylate synthase, a key enzyme in proline biosynthesis [41]. When the expression of the soybean P5CS gene was knocked-down, survival under drought stress was hampered [42]. However, a recent study comparing a drought tolerant and a drought sensitive soybean did not reveal an increase in proline level under stress, although the proline level of the tolerant cultivar was higher than that in the sensitive cultivar [43]. The involvement of proline accumulation in drought stress adjustment in soybean awaits further confirmation.

The cellular biochemical adjustment under drought stress involves the scavenging of reactive oxygen species (ROS). Under normal situation, ROS including singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical are continuously synthesized and
eliminated in plant cells as “by-products” of photosynthesis, photorespiration, and respiration in chloroplast and mitochondria [44]. Under drought stress, ROS accumulates when the production outweighs the removal [45]. The over-produced ROS will attack cellular components including nucleic acids, protein, and lipid and eventually leads to cell death [46].

ROS scavenging enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase increased in 5 soybean germplasms under drought stress [24]. The tested germplasms displayed different basal and treatment-induced level of ROS scavenging enzymatic activities, which were correlated positively to the final seed yield [24]. The study on GmPAP3 from soybean provides another example for the correlation between enhanced ROS scavenging activity and the adaptation to osmotic stress. GmPAP3 is a mitochondria localized purple acid phosphatase [47]. Ectopic expression of the GmPAP3 gene significantly reduces ROS accumulation and thereby alleviates osmotic stress [48].

Adverse environmental conditions can bring forth the misfolding of proteins that will accumulate in endoplasmic reticulum (ER) [49]. The resulting ER stress will activate unfolded protein response [49]. By global expression-profiling analyses on soybean leaves exposed to ER stress inducers and polyethylene glycol, a number of genes were identified as candidate regulatory components integrating ER stress signaling and osmotic stress responses [50]. Moreover, overexpression of soybean BiP (binding protein), an ER-resident molecular chaperone, can enhance drought tolerance in soybean [51]. This evidence tightens the link between ER stress and drought response through the activity of chaperones.

6. Molecular mechanisms of drought tolerance in soybean

In higher plants, the drought stimuli are presumably perceived by osmosensors (that are yet to be identified) and then transduced down the signaling pathways, which activate downstream drought responsive genes to display tolerance effects [52]. The tolerance involves not only the activities of protein receptors, kinases, transcription factors, and effectors but also the production of metabolites as messengers for transducing the signals. Drought tolerance is of multigenic nature, involving complex molecular mechanisms and genetic networks.

The signaling pathway of drought stress is largely overlapping with the signaling pathway of osmotic stresses which has been extensively reviewed [52]. Here, we provide a summary directly related to drought tolerance and include updated information when appropriate.

6.1. Searching for osmosensors

The perception of drought stimulus is presumably via unknown osmosensors. It is speculated that these sensors are associated with alterations in membrane porosity, integrity [53], and turgor pressure [54]. From the spatial perspective, membrane proteins, cell wall receptors, and cytosolic enzymes are all potential sensors for osmotic stress [55, 56]. For example, the families of THESEUS 1 and FERONIA receptor-like kinases (RLKs) in A. thaliana are putative stress sensors in cell wall to perceive changes in cell wall integrity and turgor pressure
On the other hand, from the functional point of view, calcium ion (Ca$^{2+}$) channels, Ca$^{2+}$ binding proteins, two-component histidine kinases, receptor-like protein kinases, G-protein coupled receptors are also potential candidates of osmosensors [60-63]. For instance, AHK1 has been postulated as a cell surface sensor that activates the high-osmolarity glycerol response 1 (HOG1) mitogen-activated kinase (MAPK) cascade in transgenic yeast. [64]

In soybean, two-component histidine kinases (GmHK07, GmHK08, GmHK09, GmHK14, GmHK15, GmHK16 and GmHK17) and receptor-like protein kinases (GmCLV1A, GmCLV1B, GmRLK1, GmRLK2, GmRLK3 and GmRLK4) have been identified as candidates of osmosensors [65-67]. However, direct evidence for their functions to perceive stress signals in soybean is still missing.

6.2. Signal transduction under drought stress

Abscisic acid (ABA) regulates the physiology (e.g. closure of stomata) and metabolism of plants (e.g. expression of enzymes) to rapidly cope with environmental challenges [68]. Biosynthesis, accumulation, and catabolism of ABA are all crucial for the transduction of ABA-mediated signals. The accumulation of ABA in response to drought is associated with the changes in the levels of Ca$^{2+}$ and ROS [60, 69]. In planta, ABA is synthesized in various cell types including root cells, parenchyma cells, and mesophyll cells. Under drought stress, ABA is transported to guard cells to control stomatal aperture [70]. ABA reaching the target tissues and cells will be recognized and the signals will be transduced down the ABA signalosome [71], including ABA receptors (PYR/PYL/RCAR), negative regulators (e.g. group A protein phosphatases 2C), and positive regulators (e.g. SnRK-type kinases).

Components of this system have been discovered in soybean. For example, GsAPK is a SnRK-type kinase from wild soybean that is up-regulated by drought stress in both leaves and roots, but down-regulated by ABA treatment in roots [72]. In vivo assay revealed that the phosphorylation activities of GsAPK is activated by ABA in a Ca$^{2+}$-independent manner, suggesting that GsAPK may play a role in the ABA-mediated signal transduction [72]. Activated SnRK-type kinases in rice and A. thaliana will phosphorylate target proteins including bZIP transcription factors and membrane ion channels [72].

Perceived stress signals may trigger transient changes in the cytosolic Ca$^{2+}$ level which acts as a second messenger [73]. Ca$^{2+}$ sensors in turn transmit and activate the signaling pathways for downstream stress responses [60]. Ca$^{2+}$ sensors include various types of Ca$^{2+}$-binding proteins: CaMs (calmodulins), CMLs (CaM-like proteins), CDPKs (Ca$^{2+}$-dependent protein kinases), and CBLs (calcineurin B-like proteins) [74]. Among these Ca$^{2+}$ sensors, all are plant and protist-specific with the exception of CaM.

Expression of the soybean CaM (GmCaM4) in transgenic A. thaliana activated a R2R3 type MYB transcription factor which in turn up-regulated several drought-responsive genes, including P5CS (encoding a proline anabonic enzyme) [75]. While the application of Ca$^{2+}$ affects the nodulelation of soybean [76], the gene encoding a soybean CaM binding protein was found to be differentially expressed in soybean nodules under drought stress [77].
The drought tolerance related CDPK family is well-studied in rice and *A. thaliana* [78, 79]. In isolated soybean symbiosome membrane, a CDPK was demonstrated to phosphorylate an aquaporin called nodulin 26 and hence enhance the water permeability of the membrane. It was hypothesized that this is an integral part of the drought tolerance mechanism [80, 81].

Besides Ca\(^{2+}\), phosphatidic acid (PA) and the intermediates of inositol metabolism are also second messengers for signal transduction [82-84]. However, there are only very limited evidence supporting the involvement of phospholipid signaling in drought stress response of soybean. The soybean nodulin gene *G93* encoding a ZR1 homologue was down-regulated under drought stress [85]. Plant ZR1 homologue such as RARF-1 in *A. thaliana* may involve in lipid signaling via interaction with phosphatidylinositol 3-phosphate [86].

When plants are subjected to drought stress, accumulation of cellular ROS will trigger the generation of hydrogen peroxide, a signaling molecule that will activate ROS scavenging mechanisms [87]. In soybean, exogenous application of hydrogen sulphide alleviates symptoms of drought stress, probably via triggering an antioxidant signaling mechanism [88].

Many studies support the roles of protein kinases in stress signaling [89, 90]. In plants, the drought responsive signal transduction of the MAPK family (MAPK, MAPKK/MEKK, MAPKKK/MKK) as well as the MAPK phosphatases (MKP) family have been relatively well-studied in *A. thaliana* and rice [89], but remained under-explored in soybean, although a PA-responsive MAPK has been identified in soybean [91].

On the other hand, some non-MAPK type protein kinases found in soybean may be related to drought responses. The soybean gene encoding a serine/threonine ABA-activated protein kinase was found to be up-regulated by ABA, Ca\(^{2+}\), and polyethylene glycol treatments [92]. The With No Lysine protein kinase 1 of soybean is another serine/threonine protein kinase that is a putative osmoregulator [93].

The ubiquitin-mediated protein degradation pathway is also an integral part of the signal transduction network [94]. This pathway directs the degradation of target proteins by the 26S proteasome and is responsive to drought stress. Two ubiquitin genes and one gene encoding ubiquitin conjugating enzyme were identified as differentially expressed genes in nodulated soybean under drought stress [77]. Overexpression of the ubiquitin ligase gene *GmUBC2* enhances drought tolerance in *A. thaliana*, via up-regulating the expression of genes encoding ion transporters (AtNHX1 and AtCLCa), a proline biosynthetic enzyme (AtP5CS), and a copper chaperone (AtCCS) [94].

### 6.3. Drought-responsive transcription factors

Transcription regulation plays an important role in drought stress response. For instance, using oligo microarray analysis, transcriptions of 4,433 and 5,098 soybean genes were found to be significantly up-regulated and down-regulated respectively when subjected to a no-irrigation period for 4 days [95]. The signal transduction pathways can ultimately regulate the expression of drought-responsive genes through diverse transcription factors. Transcription factors often target the corresponding *cis*-acting promoter elements, such as the drought stress related elements DRE, ABRE, Gbox, and T/Gbox [95, 96].
In the soybean genome, ~500 transcription factors were *in silico* annotated [96]. Increasing efforts have been placed to characterize their importance and functions in relation to drought [97-101]. Soybean transcription factors that confer drought tolerance are summarized in Table 4.

| Transcription factor | Expression under drought | Plant system used | Refs |
|----------------------|--------------------------|-------------------|------|
| GmDREB              | +                        | Wheat             | [102]|
| GmDREB2             | +                        | *A. thaliana*     | [103]|
| GmDREB3             | nc                       | *A. thaliana*     | [104]|
| GmERF3              | +                        | Tobacco           | [105]|
| GmERF4              | +                        | Tobacco           | [106]|
| GmERF089            | +                        | Tobacco           | [107]|
| GmbZIP1             | +                        | *A. thaliana*, wheat | [108]|
| GmGT-2A             | +                        | *A. thaliana*     | [109]|
| GmGT-2B             | +                        | *A. thaliana*     | [109]|
| GsZFP1              | +                        | *A. thaliana*     | [110]|
| GmWRKY54            | +                        | *A. thaliana*     | [111]|
| GmWRKY578           | nt                       | Tobacco           | [112]|

*a: up-regulated; -: down-regulated; nc: no change; nt: not tested

The transcription activity was verified by transactivation tests in yeast.

**Table 4.** Soybean transcription factors that exhibit protective function against drought in transgenic plant systems.

### 7. Strategies for breeding drought tolerant soybean cultivars

To combat water deficit, one of the most effective ways is to breed for new cultivars that exhibit durable drought tolerance. A combination of conventional breeding, marker-assisted breeding, and transgenic approaches will shed light on the crop improvement program of drought tolerance in soybean.
7.1. Conventional breeding

The high biodiversity nature of soybean allows the stacking of desirable traits through breeding. Since the genetic background of soybean germplasms varies due to spatial adaptations to diverse habitats, breeding with soybean germplasms from different origins can effectively accelerate crop improvement. A recent study suggested that wild soybean exhibited higher allelic diversity compared to cultivated soybean [113]. Since they are sexually compatible, wild soybean can potentially serve as a good genetic source in the breeding programs.

Conventional breeding could be a long and tedious process. For example, the breeding of the drought tolerant soybean cultivar Jindou 21 started by breeding Lin Xian White Soybean (an old cultivar of higher drought tolerance but lower yield) against Jindou 2 (drought tolerant, high yield, and early maturation). After the selective breeding for six generations, the resulting drought tolerance line was used as a parent of the next selection breeding and crossed with Jindou 14. The final selection breeding of Jindou 21 was carried out in the arid region of western Shanxi for seven years (1987 – 1993). Comparing to its parent Jindou 14, Jindou 21 exhibited increased yield and enhanced drought tolerant. Since then, Jindu 21 has become one of the most popularized drought tolerant soybean cultivars grown in semi-arid regions of Gansu, Ningxia, and Shanxi Provinces of China, particularly in regions where irrigated agriculture is not practical. The total cultivation area is over 3.75 million hectare [114].

7.2. Marker-assisted breeding

Drought tolerance in crops may involve different mechanisms depending on the nature of drought, making it difficult for phenotypic selection and screening through conventional breeding. A recent genomic study showed that soybean is a species of exceptionally high linkage disequilibrium (low recombination frequency) and hence marker-assisted breeding is a promising approach. The same study also identified more than 200,000 tagged SNPs for this purpose [113].

Marker-assisted breeding makes use of DNA markers that are closely linked to the target QTLs, to expedite the selection of progeny lines by replacing some time consuming phenotypic characterizations [115]. For example, delayed wilting response of canopy is associated with drought tolerance [116, 117]. Four QTLs that are associated with this trait were mapped [118], which are significantly associated with 16 SSR markers. One of the identified QTLs was identified in all tested environments which is therefore a promising candidate for marker-assisted breeding for delayed canopy wilting trait in different environments, including those with the soil type and moisture level inadequately characterized [119, 120].

7.3. Genetic engineering

With the advancement of biotechnology and availability of genomic sequence information, germplasm resources, and increasing genomic tools available for soybean research, transgenic approach has become an attractive alternative strategy in breeding. One critical hurdle
of this approach is to identify ideal candidate genes that can improve drought tolerance but do not have a yield penalty when introduced into the soybean genome.

Rapid gain-of-function experiments using heterologous model plant systems (tobacco, *A. thaliana* and rice) have been employed to screen for potential candidates. Although of lower efficiency, there are established systems of soybean transformation [121-124], allowing direct assessments of the protective functions of both native and heterologous genes in soybean.

Some promising results using this approach have been obtained, although they are all at the experimental stage. For example, AtMYB44 is a R2R3-type MYB transcription factor from *A. thaliana* that participates in the ABA-mediated abiotic stress signaling [125]. Ectopic expression of AtMYB44 in soybean led to improved drought tolerance and yet suffered from reduced growth phenotype under normal conditions [126]. Transgenic soybean expressing the *AtP5CR* gene (encoding L-Δ1-Pyrroline-5-carboxylate reductase) resulted in enhanced tolerance toward drought stress with significantly higher relative water content [127]. Introducing the *NTR1* gene from *Brassica campestris* (encoding a jasmonic acid carboxyl methyltransferase) into soybean led to increased accumulation of methyl jasmonate and enhanced tolerance toward dehydration during seed germination [128]. Overexpression of the soybean gene *GmDREB3* (encoding a dehydration-responsive element-binding transcription factor) also enhances drought tolerance, in parallel to the accumulation of proline [129].

8. Agronomic practices to alleviate the impacts of drought

While breeding programs often take a long time to complete, agronomic practices aiming at efficient use of limited water resources will give immediate results. These measures include: (1) minimizing water input; (2) reducing water loss from irrigation system and the field; and (3) increasing crop water use efficiency (WUE). In agriculture, WUE is defined as the yield of irrigated plant per total water in actual evapotranspiration (ET). A higher WUE value usually suggests a better use of water though not necessarily a higher yield [26].

Traditional irrigation systems involve open and unlined ditches channeling water from uncovered sources like wells and rivers to the fields. Besides, irrigation by flooding furrows or the whole field is common in many regions simply due to low cost [130]. A loss of more than 50% of irrigated water happens in these irrigation systems through evaporation, leakage, seepage, and percolation especially when the water source is far away from the field [131]. A well-managed pipe system can achieve 90-100% conveyance efficiency [131]. Pressurized water application methods such as advanced sprinkler and dripping systems at the terminal of the closed irrigation channels help further reduce water input [130, 132]. Sprinklers can evenly spray desirable amounts of water onto the field such that water loss through seepage and percolation can be reduced. Dripping can deliver water precisely to the root zone of the plant. This can reduce the loss of water in barren areas or consumption by weeds.

The plant at different growth stages requires different amount of water to grow and survive. ET accounts for both the evaporation and transpiration and is a measure of the amount of
water used by the crop. ET by the soybean plant roughly appears as a bell shaped curve during its life cycle. It gradually increases from the germination stage through the vegetative stage to a maximum at the early reproductive stage (R1-R2); then reduces continuously until the maturation stage [133]. The yield of soybean grown in arid regions without irrigation exhibits significant yield reduction, compared to those grown on fully irrigated land [134]. Nevertheless, delayed irrigation at flowering and early podding stages can effectively regain most of the yield as the fully irrigated plant [134-136]. Limiting irrigation to growth stages critical to the final yield can be an effective mean to reduce the input of water while water resources are scarce [137, 138].

The drought stress response of the plant involving ABA can also be used in formulating effective water saving agricultural strategies. ABA reduces stomatal aperture and hence reduces water loss through transpiration [139-142]. On the other hand, soil water deficit and water replenishment induce root growth in crops such as maize, corn [143, 144], and some soybean varieties [35]. The outgrowth of roots benefits both water and nutrient absorption upon water replenishment [145]. Based on these researches, regulated deficit irrigation (RDI) has been developed to save agricultural water by improving WUE as a result of supplying water less than the full ET of the plant. A recent study in soybean showed that compared with the fully irrigated control, irrigating with 75% water of the fully irrigated treatment could maintain over 90% of the yield while increasing the water productivity from 0.44 to 0.56 kg/m$^3$ [146].

Controlled alternate partial root-zone irrigation (CAPRI) or so-called partial root-zone drying (PRD) is a derivative of RDI. Instead of just reducing the amount of irrigation, the strategy of CAPRI is to supply water only to spatially separated parts of the root system while keeping the unirrigated parts dry [147]. The drought stress signal will be generated in the dry parts of the root system to induce growth of the whole root system and reduce stomatal aperture. On the other hand, the irrigated half of the root system will continue to absorb water to support the growth of the whole plant [145]. To prevent undesirable anatomical changes and severe damages to the root, different parts of the root system will be irrigated in turn [145]. Dripping irrigation has played an important role in CAPRI as it can precisely irrigate the desired part of the root system. Application of alternate partial root-zone drip irrigation (APRDI) has achieved promising water saving effects on different crops like cotton, grapes, and potato [148-151]. Similar strategies can be applied in soybean cultivation.

Traditional mulching involves covering of the field with straw or other harvest left-overs. The mulch can trap moisture and hence retain soil water. The degrading organic mulch also adds humus to the soil and improves the water holding capacity of the soil. In China, plastic mulch has been widely used on soybean interplanted with maize, potato or cucumber. For example, a study conducted in Shouyang County of the Shanxi Province, China suggested that mulching cultivation with hole-sowing or row-sowing techniques can increase soybean yield up to 23.4% and 50.6%, respectively [152]. Ridge-furrow mulching and whole year mulching cultivation could increase WUE by 37.3% - 58.0% and yield by 40.8% - 41.9%, respectively, in the Loess Plateau of China, compared to traditional open field cultivation [153, 154].
9. Conclusion

Soybean is nutritionally and economically important. Due to the adverse effects of agricultural drought on soybean production, drought stress in soybean has become a hot research topic. From measurement of the effect of drought on soybean to the studies of drought responsive mechanisms at morphological, physiological, and molecular level, the knowledge on drought stress and tolerance in soybean has been accumulated rapidly. With the advancement of breeding programs and agronomic practices, the production of soybean under drought can be improved by integrating all technologies and knowledge involved.

Acknowledgement

This work is supported by the Hong Kong RGC Collaborative Research Fund CUHK3/CRF/11G.

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