Challenges to design-oriented breeding of root system architecture adapted to climate change

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Roots are essential organs for capturing water and nutrients from the soil. In particular, root system architecture (RSA) determines the extent of the region of the soil where water and nutrients can be gathered. As global climate change accelerates, it will be important to improve belowground plant parts, as well as aboveground ones, because roots are front-line organs in the response to abiotic stresses such as drought, flooding, and salinity stress. However, using conventional breeding based on phenotypic selection, it is difficult to select breeding lines possessing promising RSAs to adapted to abiotic stress because roots remain hidden underground. Therefore, new breeding strategies that do not require phenotypic selection are necessary. Recent advances in molecular biology and biotechnology can be applied to the design-oriented breeding of RSA without phenotypic selection. Here I summarize recent progress in RSA ideotypes as "design" and RSA-related gene resources as "materials" that will be needed in leveraging these technologies for the RSA breeding. I also highlight the future challenges to design-oriented breeding of RSA and explore solutions to these challenges.

Key Words: *DRO1*, edaphic stress, *qSOR1*, QTL, root plasticity, root robustness.

Introduction

By 2050, the world's population is expected to grow to 9.7 to 10 billion people (Gupta *et al.* 2020). This will require an increase in food production, whereas the area of global arable land available for agriculture will not increase proportionally. In addition, the water demand for agriculture is expected to increase, even though the water supply is expected to be insufficient to meet this new demand (Gupta *et al.* 2020). Furthermore, drought and flooding events in agricultural land may also increase due to global climate change (Bailey-Serres *et al.* 2019). In order to achieve increased crop production under a cruel environment for crops, development of crops that is resistant to environmental stresses will play an important role.

Since the "Green Revolution" has developed high-yield varieties of rice and wheat, crop breeding for maximum yield in an environment where water and fertilizer are available has primarily focused on improving aboveground plant architecture (Khush 2001). However, it is also important to improve belowground plant architecture to facilitate future increases in crop production in unstable environments where water and nutrients are insufficient (Herder

et al. 2010). Many abiotic stresses such as drought, submergence, and nutrient deficiency or excess affect plants through their roots. However, little progress has been made in improving root traits compared to improving aboveground traits.

Crossbreeding has been a main driver of crop development to this date. Breeders' intuition and experience has greatly contributed to the selection of promising breeding lines after crossing (**Fig. 1A**). Unfortunately, in many cases, breeders cannot directly visualize roots because they remain hidden underground. To overcome this limitation, many researchers are developing technologies that enable high-throughput phenotyping of root traits in the field (Atkinson *et al.* 2019). At present, there are few low-cost, high-throughput root phenotyping methods available for breeding of root characters in the field (Teramoto *et al.* 2019, Yoshino *et al.* 2019). Shovelomics, a method consisting of digging up the root base of plants grown in the field and measuring root characters, is one of the simplest and easiest root phenotyping methods (Trachsel *et al.* 2011), but provides only limited information on the basal parts of the root, not a picture of the whole root system. Thus, it is still difficult to improve root traits by phenotypic selection during the breeding process.

How can root traits be improved in an efficient manner? Recent advances in molecular biology and biotechnology have provided many breeding techniques for practical crop breeding, such as DNA marker-assisted selection, genomic

A

Conventional breeding

Social needs
⬇
Breeding target
⬇
Option of elemental technologies
(genetic resources, crossbreeding)
⬇
Crossing
⬇
Selection of promising lines
(by intuition and experience of the
breeders, sometimes by chance)
⬇
Field trials
⬇
Seed production
⬇
Release of new variety

Crossbreeding is not reproducible

B

Product development

Social needs
⬇
Development target
⬇
Option of elemental technologies
(design, materials, methods)
⬇
Development of
missing elemental
technologies
⬇
Making & testing of prototype
⬇
Mass production
⬇
Release of new product

C

Design-oriented breeding

Social needs
⬇
Breeding target
⬇
Option of elemental technologies
(design, gene resources, methods)
⬇
Development of
missing elemental
technologies
⬇
Making & testing of plant lines
⬇
Seed production
⬇
Release of new variety
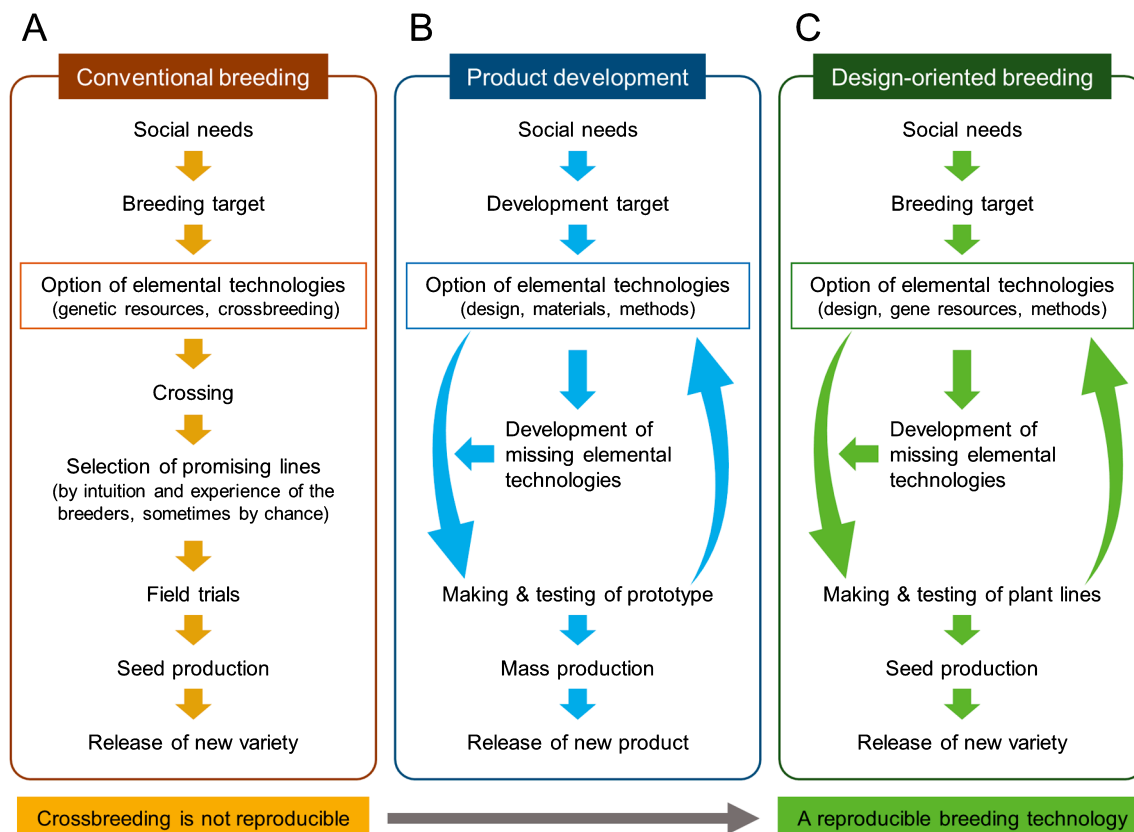
A reproducible breeding technology

Fig. 1. Concept of design-oriented breeding based on the different features between crossbreeding and industrial product development.

selection, and genome editing (Hickey *et al.* 2019). These techniques are expected to be used for the breeding of root traits that are difficult to conduct phenotypic selection in the field. To do so, a new breeding strategy that does not require root phenotypic selection will be needed. An ideal breeding without phenotypic selection is to design an ideal root system adapted to a target edaphic environment via modification of the genes associated with target root traits.

The developmental process used in industrial manufacturing may be helpful in considering how to conceptualize such a breeding strategy. In developing an industrial product, a prototype product is designed to fulfill a social need, and optimal materials and production methods are selected before the prototype is developed (**Fig. 1B**). When a required technology is missing, it must be developed before production of the prototype can begin. When the performance of the prototype is acceptable, it is mass produced and then released. Compared to product development, a major problem with conventional breeding is that a common ideotype—i.e., the "design"—is not agreed upon by stakeholders beforehand. Crop design is often done independently by each breeder, and promising breeding lines are likely to be selected by the judgment of individual breeders (**Fig. 1A**). This process lacks reproducibility. Another problem is that the effects of genetic resources—i.e., the "materials"—can be inconsistent. In crop breeding, a gene that shows an effect on a target trait in one variety

will not necessarily have the same effect in another. In contrast, during product development, the same elemental technologies can be used to produce a given product anywhere, with high reproducibility.

Like product development, protocols of design-oriented breeding can be divided into three elemental components: "design", "materials", and "methods" (**Fig. 1C**). Each of these three elements can be understood to correspond to different features of a plant breeding system: "design" corresponds an ideotype, "materials" to gene resources, and "methods" to various breeding techniques, including DNA marker-assisted selection, genomic selection, and genome editing, among others. Of these components, breeding techniques are highly reproducible, although some techniques (e.g., tissue culture) may require modification depending on the species and/or subspecies used. On the other hand, ideotypes and gene resources are not highly reusable for different species. If they could be as consistently applied across species as breeding techniques can be, this would facilitate the development of new crops efficiently by strategies that did not focus on phenotypic selection (**Fig. 1C**).

In this article, I consider the design-oriented breeding of roots, and summarize the current status and challenges for each elemental technology related to plant root breeding. I focus on "design" and "materials" because "methods" are commonly considered by researchers interested in other,

non-root traits. Root traits include root system architecture (RSA), root structure, physiological function, and root-microbe interactions, and it is difficult to cover each of these in a single review. Therefore, I focus only on RSA, which is the main component determining the area from which roots are able to capture water and nutrients from the soil (Kitomi *et al.* 2018).

Design: what is the plant ideotype with respect to root system architecture?

During crop breeding, each breeder imagines society's needs in the present or for the future and selects promising lines that have the required traits. The ideotype is a very useful conceptual framework for breeders interested in phenotypic selection. Since the "Green Revolution", the breeding of wheat and rice for aboveground architectural traits has progressed with the semi-dwarf plant as an ideotype (Khush 2001). Like aboveground architecture, RSA ideotypes have also been proposed by many researchers (Lynch 2019, Meister *et al.* 2014, Schmidt and Gaudin 2017). However, RSA ideotype breeding is not widely practiced

because roots remain hidden underground. In this section, I summarize the representative RSA ideotypes proposed by previous studies and discuss the challenges facing targeted breeding of RSA ideotypes. I also consider two types of soil-related stress: biotic stresses, such as pests and diseases, as well as abiotic stresses, such as drought and salinity. I focus on the main abiotic stresses relevant for agriculture, especially water stresses, such as drought and flooding, and mineral stress, such as nitrogen, phosphorus, and salinity. I introduce the RSA ideotypes by comparing these abiotic stresses in two agroecosystems: regular fields (including both dry fields and upland fields), and paddies.

Drought stress

When rainfall is scarce, continued water evaporation from the soil surface results in drought conditions. Relative to the RSA suitable during normal conditions (**Fig. 2A**), a deeper RSA may be more advantageous under drought conditions brought about by drying from the soil surface (**Fig. 2B**). Under the surface, those maize varieties with fewer crown and lateral roots, as well as steeper and thicker roots, are better adapted for water uptake from the subsoil
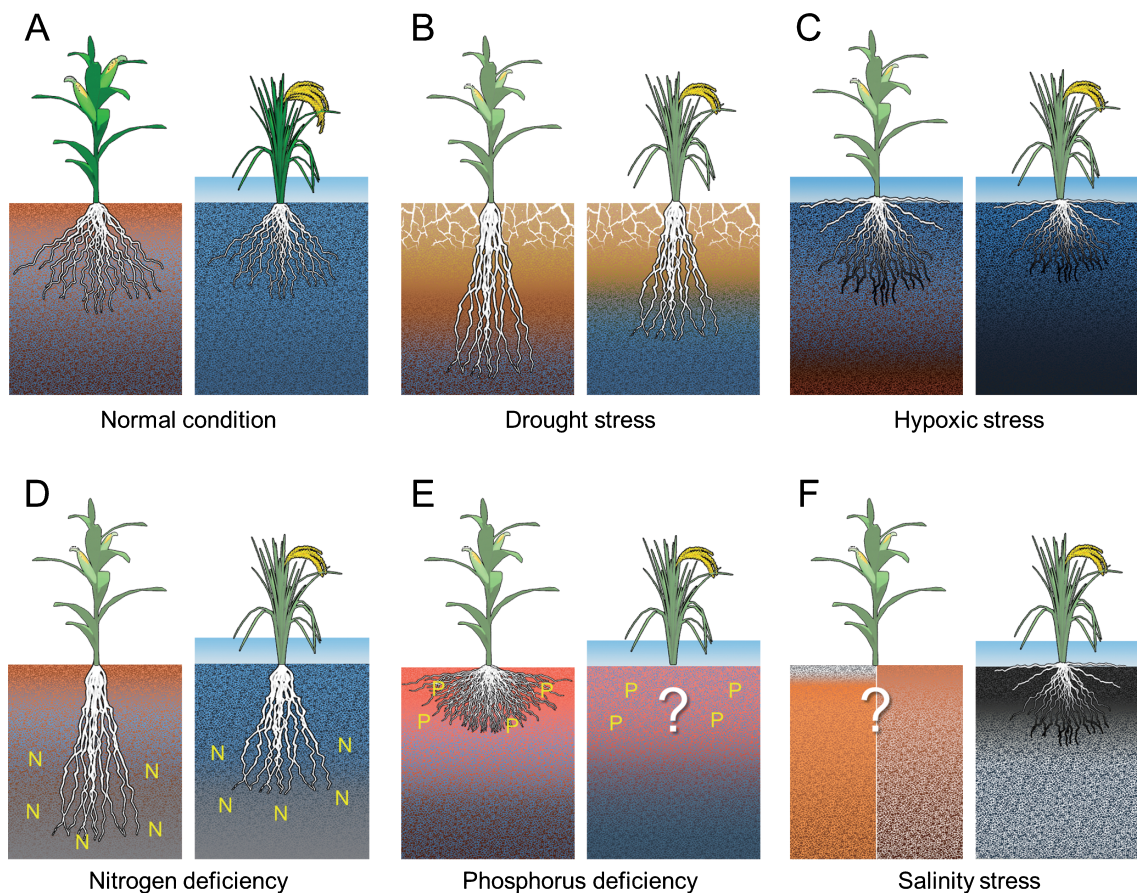

Fig. 2. Promising ideotypes of root system architectures adapted to abiotic stresses. In each panel A–F, left and right figures represent regular and paddy fields, respectively. Each panel shows schematic models of the ideal root system architecture under a different abiotic stress condition. Plants shown are maize and rice—i.e., representative monocot crops—grown in regular and paddy fields, respectively. N, nitrogen; P, phosphorous. Question marks in the figure mean that no RSA ideotype have been established based on field studies.

(Lynch 2013, Lynch and Wojciechowski 2015). In rice, a deeper rooting phenotype is related to a functional allele at *DEEPER ROOTING 1* (*DRO1*), a quantitative trait locus (QTL) for root growth angle, which limits the impact of drought stress. A comparative study found that a functional *DRO1* allele results in increased grain yield, whereas the shallow rooting phenotype resulting from a non-functional allele at *DRO1* was susceptible to drought stress (Uga *et al.* 2013). Paddy fields consist of an impermeable hardpan under a plow layer, depending on soil condition. It has been thought that the ability of roots to penetrate the hardpan and for lateral root formation to efficiently capture water from the plow layer are important plant adaptations for paddy crops (Kano-Nakata *et al.* 2013, Suralta *et al.* 2018). However, in many paddies in drought-prone areas around South Asia, this hardpan is not formed (Singh *et al.* 2017). For those paddies without a hardpan layer, the RSA ideotype idea for avoiding drought stress may be the same as that for regular fields (**Fig. 2B**).

Hypoxic stress caused by flooding

In extreme submergences, such as catastrophic floods, the roots can act as an anchor to prevent a plant from being washed away. When flooding occurs from extensive precipitation such that water covers the soil surface, field crops will experience stress disorders, such as root rot caused by hypoxic stress. In this case, rooting on the soil surface and/or adventitious rooting around the topsoil may be an effective RSA to avoid hypoxic condition (**Fig. 2C**; Pedersen *et al.* 2021). While common maize and its wild relatives (teosintes) are susceptible to flooding, some teosinte accessions found in the wetlands of Nicaragua have been found to be adapted to flooding conditions (Mano and Omori 2007). These accessions can develop soil-surface roots (SOR) to access oxygen from the air, even when the plant is flooded. When flooding conditions continue until harvest in paddies without drainage, soil reduction can be caused by hypoxic conditions, resulting in reduced root development (Fageria *et al.* 2008, Takai and Kamura 1966). In rice, the SOR phenotype has been also found in Indonesian lowland rice belonging to the Bulu ecotype, although common rice ordinarily develops underground roots (Ueno and Sato 1989). SOR formation may have arisen because selection pressures have driven the Bulu ecotype to adapt to severe hypoxic environments (Lafitte *et al.* 2001). Recently, a QTL associated with SOR formation, *quantitative trait locus for SOIL SURFACE ROOTING 1* (*qSOR1*), has been isolated from one of these Bulu varieties (Kitomi *et al.* 2020). In a paddy field subject to reducing stress, rice plants with SOR, caused by a loss-of-function allele at *qSOR*, had higher grain yields than rice without SOR. Thus, regardless of the crop species, the SOR phenotype may be an effective RSA in avoiding hypoxic stress (**Fig. 2C**).

Nutrient deficiency

There are two types of minerals found in field conditions: water-soluble and water-insoluble minerals. Water-soluble minerals tend to move into the subsoil according to gravity, following water, whereas water-insoluble minerals tend to remain in the topsoil. Of the most important minerals for plant growth, nitrogen (N) is mobile and phosphorus (P) is immobile. Since both minerals are very important for crop production, they are typically added to the fields in large quantities in developed regions. On the other hand, in developing regions, the soil may be deficient in both minerals due to lack of adequate fertilizer application. In both cases, RSA optimization is important because it allows more efficient absorption of each mineral.

In the field, N easily shifts to the subsoil with water. For this reason, RSAs consisting of steeper, longer, fewer, and thicker roots may be most effective for field crop N accumulation in (**Fig. 2D**). For example, a "steep, cheap, and deep" RSA ideotype for maize has been proposed; this would permit effective uptake of N as well as water from the subsoil (Lynch 2013). In paddy fields with a hardpan under the plow layer, N loss caused by leaching to the subsoil is negligible (Yamamuro 1986). However, deep roots have been reported to affect to yield. In rice, a deep root-type line, showing higher N uptake after the heading stage, had a higher yield compared to a shallow root-type line (Arai-Sanoh *et al.* 2014). Just as in regular fields, a deep RSA may also be advantageous for N absorption in paddy fields (**Fig. 2D**).

An RSA ideotype including shallower axial roots, enhanced adventitious rooting, a greater number of axial roots, and a greater dispersion of lateral roots may more effectively capture P from the topsoil under low P soil conditions (**Fig. 2E**; Lynch 2011). Common beans (*Phaseolus vulgaris*) showing a shallow RSA phenotype were found to show higher fertility than deep RSA ones in P-deficient soils (Lynch and Brown 2001). The same trend was also observed in maize (Lynch 2019, Zhu *et al.* 2005). In rice, P deficiency is a major limiting factor in upland and rain-fed lowland fields in several developing regions (Ismail *et al.* 2007). P treatments applied to paddy fields behave similar those applied to regular fields (Oo *et al.* 2020). There, rice roots have been found to develop in soil zones characterized by high P concentration (He *et al.* 2003). These data suggest that shallower RSAs may be advantageous in paddy fields with P deficiency condition just as they are in regular fields (**Fig. 2E**). However, which RSA ideotype is suitable for P-deficient paddy fields remains unclear and requires further study.

Salinity stress

Salinity stress is one of major abiotic stresses and is expected to have an adverse impact on an estimated 50% of all arable lands worldwide by 2050 (Butcher *et al.* 2016). To develop salt-tolerant crops, many studies have identified mechanisms of salt tolerance and to isolate related genes

(Bailey-Serres *et al.* 2019, van Zelm *et al.* 2020). However, no RSA ideotype for salt resistance has been reported to date. If such an RSA existed, it would be expected to enable stable crop production in saline-prone areas, which are estimated to increase in abundance in the future (Rogers and Benfey 2015). Salts present in fertilizer and/or irrigation water can accumulate in the soil surface or subsoil, and the degree to which this happens depends on differences in cultivation conditions including the natural environment and irrigation (Ismail *et al.* 2007, Munns *et al.* 2006). Little is known about the ideal RSA for such conditions (**Fig. 2F**), although inducible development of adventitious roots with cortical aerenchyma may be useful in irrigated saline soils where water input is available (Schmidt and Gaudin 2017). In coastal areas, saltwater intrusion has resulted from sea level rises caused by global warming, resulting in saline damage to crop production (Gopalakrishnan *et al.* 2019). In coastal paddy fields, long-term saline condition cause not only salinity stress but also reducing stress due to soil structural changes by salts (Qadir and Schubert 2002, Srivastava *et al.* 2014). Recently, the SOR phenotype has been found to allow rice plants to avoid reducing stress caused by saline conditions (Kitomi *et al.* 2020). This suggests that the SOR phenotype may be useful in saline paddy fields (**Fig. 2F**) as well as in flooded paddy fields (**Fig. 2C**), although the SOR does not strengthen ability of salinity tolerance (Kitomi *et al.* 2020). The degree to which the SOR phenotype is effective in reducing salt stress in field crops should be clarified in future studies.

Robustness vs plasticity in RSA

Plants have both robustness- and plasticity-oriented responses to environmental changes. With respect to roots, robustness is understood as the ability to continue innate development irrespective of environmental changes, whereas plasticity reflects the ability of the root to adapt to these changes. In the following section, I discuss the advantages and disadvantages of root robustness and plasticity for RSA in response to a hypothetical environmental change to the water environment.

When soil moisture varies strongly over short time scales in the field, a robust RSA would be insensitive to short-lived changes, thereby reducing the cost of mounting a response to environmental variation. On the other hand, a plastic RSA would be sensitive to environmental changes and would involve an adaptive strategy. The extra costs associated with adapting to constantly changing environments may result in decreased crop yield (Schneider and Lynch 2020). With respect to long-term environmental changes, the robust RSA strategy will have an advantage if it is well adapted to the environment. However, if the RSA type is not well adapted to the target environment, plant growth will be compromised by abiotic stress. For example, deep-rooted cultivars could avoid drought while shallow-rooted cultivars would have limited growth (Uga *et al.*

2013). However, when the deep root-type cultivar is grown under normal moisture conditions, the development of roots that extend to the subsoil without fertilization will be costly and will not enhance seed production. For example, deep rooted upland rice generally has a lower yield than shallow rooted lowland rice (Atlin *et al.* 2006, Fukai and Cooper 1995). In contrast, the plastic RSA strategy allows plants to response to stress conditions (e.g., drought) by adaptively changing their roots only when drought is experienced. For example, in rice, an introgression line showing plasticity in lateral root length and density under drought conditions showed increased shoot biomass relative to the wild type variety, which lacked RSA plasticity (Kano-Nakata *et al.* 2013). Thus RSA plasticity gives plants the ability to adapt to the environment, but only at a cost (Schneider and Lynch 2020), and it is not well understood whether RSA plasticity contributes to increase crop yield under stress conditions.

When considering RSA ideotype breeding, it is important to first ask which is easier to use: a robust RSA or plastic RSA? At present, robust RSAs may be preferable. Ideal plant architecture (IPA) for aboveground plant parts is usually determined by robust traits such as culm and panicle size and number (Dong *et al.* 2017, Jiao *et al.* 2010). These IPAs are easy to select for ideotype breeding in practice. A robust RSA is probably also desirable for well-defined climates, with distinct wet and dry seasons. For example, deep root RSA phenotypes would be useful during prolonged drought, while shallow root RSA phenotypes would be useful in lowland fields prone to flooding. In contrast, there are few comprehensive field studies that have identified how plasticity may contribute to crop productivity and which morphological changes are advantageous (Schneider and Lynch 2020). Therefore, the method by which RSA plasticity can be utilized as an ideotype is not well understood. However, if the mechanisms responsible for RSA plasticity are elucidated in the field, it could find use as a new ideotype in the future.

Beyond the relationship between different abiotic stresses and RSA, there are other factors that should be considered to allow for RSA ideotype breeding. High temperature stress often occurs simultaneously in environments where drought and salinity stresses are also present (Calleja-Cabrera *et al.* 2020). In such cases, it is necessary to select RSA profiles that show high resistance against multiple abiotic stresses. It is also possible that N and P deficiencies may occur simultaneously in some regions. In these cases, since the RSA ideotypes required for N and P deficiencies are completely different (Lynch 2019), breeders will have to determine whether deep, intermediate, or shallow root phenotypes would be prioritized. Thus, there is no universal RSA ideotype for multiple abiotic stresses (Lynch 2019, Schneider and Lynch 2020). When considering RSA ideotypes, the influence of cropping system, including factors such as planting density (Meister *et al.* 2014), cultivation period (Voss-Fels *et al.* 2018), water source (i.e., whether crops are irrigated or rainfed)

(Schmidt and Gaudin 2017) and of soil physical properties such as soil compaction (Ramalingam *et al.* 2017) should also be considered. Data collection in all environments and for all cropping systems, is an unrealistic goal. For this reason, simulation studies that model RSA phenotypes can complement field-based RSA research (de Dorlodot *et al.* 2007, Postma *et al.* 2014, Rellán-Álvarez *et al.* 2016). With the accumulation of such data, accurate simulation-based design of RSA ideotypes will be possible in the future.

Materials: gene resources to enable design-oriented breeding

Breeding materials are essential for crop development. Plant crossbreeding involves selecting accessions with the desired characters and using them as breeding stock to produce new plants with novel phenotypes. On the other hand, recently developed breeding techniques, such as DNA marker-assisted selection and genome editing, cannot use directly such existing genetic resources as breeding material. For example, DNA marker-assisted selection requires the mapping of genes related to the target trait before existing genetic resources can be used as breeding materials. Moreover, the cloning and characterizing of genes responsible for target traits is required before genome editing can take place. So far, many promising RSA-related genes have been identified in crops. These discoveries have resulted from many different genetic approaches, including mutant assays, QTL analysis, and genome-wide association studies (Deja-Muylle *et al.* 2020, Meister *et al.* 2014, Rogers and Benfey 2015). Moreover, the molecular functions of RSA-related genes in maize and rice have been described in detail (Hochholdinger *et al.* 2018, Kitomi *et al.* 2018, Wachsman *et al.* 2015). Only a few RSA-related cloned genes have been shown to contribute to abiotic stress resistance under field conditions (Mickelbart *et al.* 2015). In this section, genes related to robust and plastic RSAs, which we could use as breeding materials, are introduced.

Robust RSA-related genes

Some robust RSA-related genes, including *DRO1* and *qSOR1*, have been shown to contribute to resistance to abiotic stress in the field. *DRO1* is the first cloned QTL for root growth angle in crops (Uga *et al.* 2013). This QTL was identified on rice chromosome 9 from a mapping population derived from a cross between 'IR64', lowland rice with a shallow root type, and 'Kinandang Patong', an upland rice with a deep root type (Uga *et al.* 2011). An near-isogenic line (NIL) of 'IR64' introducing a functional allele at *DRO1* from 'Kinandang Patong' showed deeper rooting than 'IR64', resulting in higher yield under drought conditions in upland fields (Uga *et al.* 2013) and paddy fields (Uga *et al.* unpublished data). *qSOR1* is the second cloned QTL for root growth angle in crops (Kitomi *et al.* 2020). This gene has been identified on rice chromosome 7 from a mapping population derived from a cross between

'Gemdjah Beton', a lowland rice with SOR, and 'Sasa-nishiki', a lowland rice without SOR (Uga *et al.* 2012). A NIL of 'Sasanishiki' containing a non-functional allele at *qSOR1* from 'Gemdjah Beton' showed SOR in both upland and paddy fields (Kitomi *et al.* 2020). This NIL with SOR avoids the reducing stress found in saline paddy fields and results in reduced yield loss.

Homology searches based on the amino acid sequence of *qSOR1* revealed that rice *DRO1* was most closely related to rice *qSOR1* (Kitomi *et al.* 2020). There are several regions of conserved sequence between these two genes, although the overall similarity of their amino acid sequences is low (i.e., less than 30%). Recent work has indicated that there are four *DRO1* homologs in rice, which constitute three subgroups (Kitomi *et al.* 2020), named *DRO1*, *qSOR1/DRL1* (*DRO1-like 1*), and *DRL2*, respectively. A study of *DRL2* mutant lines made by genome editing demonstrated that *DRL2* was also involved in root growth angle (Kitomi *et al.* 2020). Taken together, these data suggest that the three subgroups of the *DRO1* family, have important roles in regulating RSA in rice.

Laboratory studies have shown that both *DRO1* and *qSOR1* are early auxin response genes involved in root gravitropism in rice (**Fig. 3**, Kitomi *et al.* 2020, Uga *et al.* 2013). In natural conditions, both genes show robust RSA phenotypes in both regular and paddy field environments but do not affect tiller angle (Kitomi *et al.* 2020). Furthermore, they have little effect on shoot and root morphology other than on root growth angle. Combining the effects of both genes may make it possible to produce a wide variation of RSAs, without considering investment trade-offs between shoots and roots. Root trait breeders have often
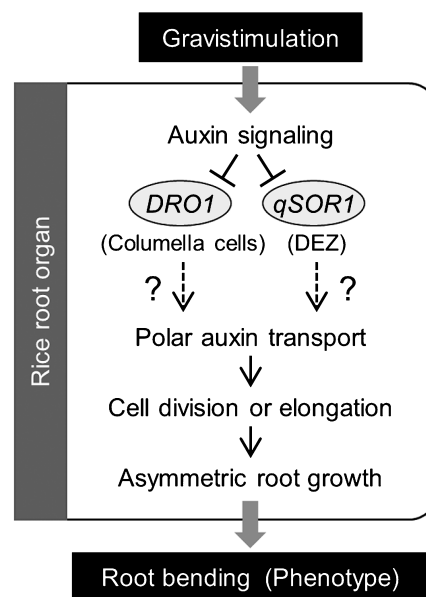

Fig. 3. Proposed regulatory pathways of gravity-induced root bending via *DRO1* homologs in rice. Term in parentheses indicates the major tissues expressing each gene. DEZ, distal elongation zone.

assumed that it would be difficult to modify only target root traits without simultaneously affecting shoot characters because of this trade-off (Voss-Fels *et al.* 2017). However, our studies have demonstrated that it is possible to improve RSA without considering the trade-off between shoots and roots, although the effect of each gene must be carefully characterized in a field environment before being used for breeding.

DRO1 homologs have also been reported to be involved in root gravitropism in dicots, including species in the genera Medicago, Arabidopsis, and Prunus (Ge and Chen 2016, Guseman *et al.* 2017, Taniguchi *et al.* 2017). This suggests that *DRO1* homologs may form an important gene family regulating RSA across the angiosperms. If so, the *DRO1* family is likely to be used as a gene resource for RSA breeding in crops other than rice. In what follows, I will summarize the *DRO1* family members that have been identified thus far.

Among monocots other than rice, *DRO1* homologs have also been identified in wheat, although whether they affect RSA is still unknown (Ashraf *et al.* 2019). In 2007, *LAZY1*, a gene involved in shoot gravitropism, was identified in a rice mutant (Yoshihara and Iino 2007). More recent studies have found that *LAZY1* is highly similar to the *DRO1* family in multiple domain sequences (Guseman *et al.* 2017), including a conserved C-terminal domain known as conserved C terminus in the LAZY1 family of proteins (CCL) (Taniguchi *et al.* 2017). *Tiller Angle Control 1* (*TAC1*), a gene involved in tiller angle in rice (Yu *et al.* 2007), has also been found to have a high similarity to the *DRO1* family in multiple domain sequences without a CCL domain (Nakamura *et al.* 2019). These data further suggest that *DRO1* homologs form an extensive gene family associated with shoot and/or root gravitropism in monocots.

In dicots, several *DRO1* homologs have been found in aberrant gravitropic mutants. In Medicago, a gene known as *NEGATIVE GRAVITROPIC RESPONSE OF ROOTS* (*NGR*) was isolated from mutants showing roots emerging above the soil surface (Ge and Chen 2016). In Arabidopsis, *AtDRO1* mutants showed shallower lateral root angles compared to wild type plants (Guseman *et al.* 2017). *LAZY1* homologs have also been found in Arabidopsis (Taniguchi *et al.* 2017), and each of these could be classified into one of three groups (Nakamura *et al.* 2019): (1) *LZY1* (*LAZY1-LIKE 1*), a rice *LAZY* homolog, is involved in shoot gravitropism; (2) *LZY4* is involved solely in root gravitropism; and (3) *LZY2* and *LZY3* are involved in both shoot and root gravitropism. Nakamura *et al.* (2019) mentioned that *LZY3* is a rice *DRO1* homolog. However, phylogenic analysis of the *DRO1* gene family in both monocots and dicots revealed that many dicots showed more similar sequences to *qSOR1* than to *DRO1*, suggesting that the *qSOR1* sequence may be more common than the *DRO1* sequence in angiosperms (Kitomi *et al.* 2020).

The homologs of *DRO1* and *LAZY1* are therefore present in both monocots and dicots, forming a large gene family

that controls shoot and root gravitropism (Kitomi *et al.* 2020). In Arabidopsis, the CCL domain of the LZY protein binds to the Brevis radix (BRX) domains of the RCC-like domain (RLD) protein, thereby allowing LZY to transport RLD from the cytoplasm to the plasma membrane (Furutani *et al.* 2020). Furutani *et al.* (2020) showed that these genes affected gravitropism through their involvement in PIN-FORMED (PIN)-mediated auxin polarity transport. Recently, light signals were found to regulate *LZY* expression, resulting in change of response to the gravistimulus (Yang *et al.* 2020a). Light promotes accumulation of ELONGATED HYPOCOTYL5 (HY5) proteins, which directly bind and activate the expression of *LAZY4/ LZY3/NGR2/DRO1* in Arabidopsis roots. Thus, further clarification of the functions of individual genes in the *DRO1/ LAZY1* family is expected to enable more precise genetic control of the above- and belowground architecture of crops. In other words, *DRO1/LAZY1* family genes have strong potential to be used as "materials" for design-oriented breeding of not only the RSA but of the architecture of the whole plant in many crops.

Plastic RSA-related genes

As for robust RSAs, few genes involved in plastic RSAs have been reported. However, genes involved in plastic RSAs may be just as important a breeding material as genes involved in robust RSAs (Dwivedi *et al.* 2020). In rice, *Rice Morphology Determinant* (*RMD*), a gene encoding an actin-binding protein, controls gravitropic responses to low P condition (Huang *et al.* 2018). Since RMD controls from deep to shallow rooting at low P conditions, RMD is expected to be a useful RSA gene, enhancing P capture under low P conditions. However, its usefulness has not been demonstrated in the field, so further studies designed to verify this effect are needed. The paucity of reports on the usability of genes for plastic RSAs may be due to the fact that it is difficult to evaluate them in the field, since it is difficult to control stress conditions underground. Another reason may be related to the crop domestication process. During domestication, humans may have selected mainly for yield-related traits, including seed size and number, as well as cultivation-related traits including seed shattering and dormancy. In cultivated fields where domesticated crops are more stable than they were in their natural habitat (Wallace *et al.* 2018), plastic RSA responses may have been unwanted and ultimately lost by the modern farming system (Calleja-Cabrera *et al.* 2020). Consequently, genetic diversity for RSAs in modern crops was likely reduced by domestication and breeding bottlenecks (Voss-Fels *et al.* 2018). Placido *et al.* (2020) reported that allele of *Agropyron elongatum*, a wild relative of wheat, at *LATERAL ROOT DENSITY* (*LRD*), a gene involved in lateral root formation, promoted lateral root growth under drought conditions. Crop wild species are a promising genetic resource for future breeding of improved root stress response (Calleja-Cabrera *et al.* 2020). Therefore, the

discovery of useful alleles from genetic resources such as wild species is important for the design-oriented breeding of RSAs in the future.

Future perspective: is the design-oriented breeding of RSA possible?

In this review, I have described two elemental technologies ("design" and "materials") for design-oriented breeding of RSA. Although RSA ideotypes have been proposed in many crops, few RSA ideotypes adapted to abiotic stresses have been established based on individual genes for RSA-related traits whose functions have been characterized in field studies (Arai-Sanoh *et al.* 2014, Kitomi *et al.* 2020, Uga *et al.* 2013, 2015). Bi-parental mapping populations, such as recombinant inbred lines, are not suitable for evaluating the effects of each trait because such populations include lines showing simultaneously varying phenotypic segregations (Yamamoto *et al.* 2014). However, plant materials with unified genetic backgrounds, such as NILs and gene pyramiding lines, may be better options to examine these traits. However, the isolation of genes or QTLs involved in target traits and the development of plant materials such as NILs are time-consuming. With respect to the "materials" available to study RSA ideotypes, a large number of RSA-related genes have been identified, but their effects on target traits in the field have not been adequately validated. This is also a time-consuming activity, and many individual genes isolated from different accessions should be evaluated in the same genetic background.

A common challenge with both elemental technologies is that the development of plant materials needed to evaluate target traits or genes is a significant time investment. Two techniques that have been developed in recent years that may help alleviate this problem. The first is genome editing. For example, multiple RSA-related genes found in different species or varieties can be easily introduced into a model or representative accession by genome editing techniques. The effect of each gene on the different environmental stresses (G × E) can then be evaluated in the same genetic background. By simultaneously editing multiple genes, we can also verify the effect of gene pyramiding on different environmental stresses (G × G × E). In addition, an allele gained by genome editing will allow us to identify the allelic variation involved in phenotypic variation. The second technique is speed breeding (Hickey *et al.* 2019, Watson *et al.* 2018). Speed breeding permits the shortening of plant generation time by controlling plant environments, resulting in rapid accumulation of genes or QTLs. Thus, both techniques can be used to collect information relevant to ideotype "design" and "materials" in a short period of time.

Another challenge with both elemental technologies is that high-throughput RSA phenotyping in the field is very difficult and labor-intensive (Yoshino *et al.* 2019). In particular, when evaluating root plasticity under abiotic stress conditions, it is necessary to observe RSA development continuously. However, this is still difficult to do in the field (Voss-Fels *et al.* 2018). Currently, phenotyping platforms permitting the controlling and monitoring of environmental conditions in greenhouses and/or growth chambers have been developing (Yang *et al.* 2020b). In addition, non-invasive RSA phenotyping techniques using X-ray computed tomography and magnetic resonance imaging are also being developed (Atkinson *et al.* 2019, Teramoto *et al.* 2020). Combining these technologies would permit the collection of highly reproducible RSA data non-destructively under reproducible environmental stress conditions and would be a viable alternative to evaluating plants in the field. I believe that progress in the research described above will make the design-oriented breeding of RSAs a reality.

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