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Tuber, or not tuber: Molecular and morphological basis of underground storage organ development

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Abstract

Underground storage organs occur in phylogenetically diverse plant taxa and arise from multiple tissue types including roots and stems. Thickening growth allows underground storage organs to accommodate carbohydrates and other nutrients and requires proliferation at various lateral meristems followed by cell expansion. The WOX-CLE module regulates thickening growth via the vascular cambium in several eudicot systems, but the molecular mechanisms of proliferation at other lateral meristems are not well understood. In potato, onion, and other systems, members of the phosphatidylethanolamine-binding protein (PEBP) gene family induce underground storage organ development in response to photoperiod cues. While molecular mechanisms of tuber development in potato are well understood, we lack detailed mechanistic knowledge for the extensive morphological and taxonomic diversity of underground storage organs in plants.

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Introduction

Underground storage organs (USOs) are a solution to the problem of inconsistent resource availability for plants, providing reserves during periods of unfavorable conditions, dormancy, and ecological disturbance. Plants with USOs accumulate large stocks of water and nutrients (e.g. carbohydrates) in a variety of modified organs like leaf bases, stems, and roots (Box 1). These resources allow plants to jumpstart regrowth after seasonal or unexpected changes in their environment with less dependence on external resources [1]. Further, buds located on some USOs are protected from aboveground stressors and can give rise to new aboveground shoots when conditions improve [2]. These adaptations allow plants with USOs to thrive in drier, cooler, and more seasonal climates [3] and diversify at higher rates relative to plants without USOs [4]. Despite their ecological importance and key role in staple food crops, the molecular mechanisms and processes that generate such diversity are unknown across large parts of the plant tree of life.

USOs have evolved repeatedly in vascular plants and display extensive phenotypic diversity (Box 1) [5]. These macromorphologies can originate from stem, root, leaf, or hypocotyl tissues, and they may develop either through shared or distinct developmental and molecular processes. USOs undergo **thickening growth** through the proliferation of various lateral meristems, followed by cell expansion to accommodate starch and other stored carbohydrates. This thickening growth is initiated by regulatory genes that are downstream of environmental signals, allowing USO **induction** to be integrated with seasonal cues (Figure 1). While the molecular mechanism of stem tuber development is well-characterized in potato, we lack a comparable understanding in other taxa and types of USOs. Below, we review evidence for shared molecular processes underlying thickening and induction in diverse angiosperm taxa, emphasizing advances in the last two years, and

Box 1. Underground storage organs (USOs) briefly defined (See the study by Tribble et al. [5] for more detailed descriptions and examples)

- Bulb: a **vertically compressed stem** in which apical and axillary buds are surrounded by **concentric layers of enlarged leaf bases,** which are the primary storage organs.
- Corm: similar to bulbs in their **vertically oriented growth**, but the **stem** tissue serves as storage and stored reserves are uniformly distributed.
- Rhizome: **horizontally growing stems** that grow somewhat uniformly in diameter for storage, and from which aboveground and belowground branches may develop at nodes.
- Root tuber: **roots** evenly thickened along their entire length or roots enlarged at the tip serve as storage organs, and are wider than fibrous roots on the same plant.
- Stem tuber: belowground regions of the **stem** that enlarge for storage; unlike corms and rhizomes, storage is not uniformly distributed along the length of the belowground stem.
- Swollen hypocotyl: the intermediary region between the first cotyledon and the belowground radicle or root.
- Taproot: a single straight, vertical **root** that enlarges for storage, which is sometimes contiguous with a swollen hypocotyl.

discuss outstanding questions about the genetic basis of USO development and morphology.

Forms of thickening growth in USO development

Primary thickening growth

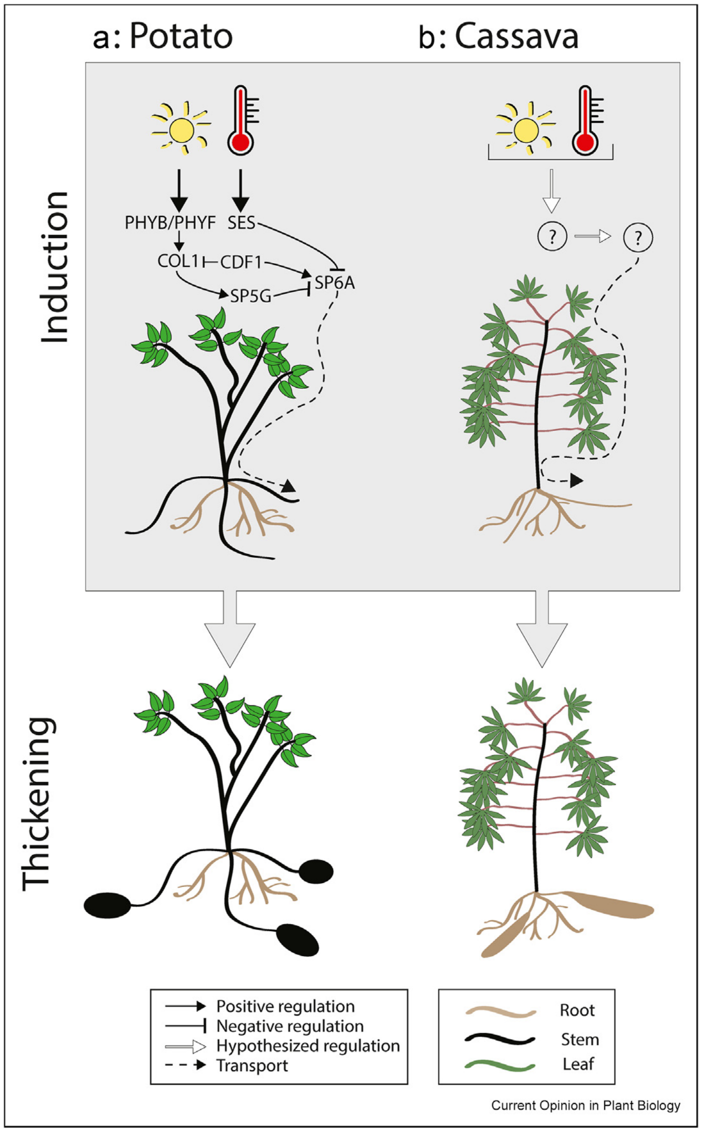
Plant growth is traditionally dichotomized into a primary growth phase and secondary growth phase. Primary growth is typically synonymized with elongation of the stem while secondary growth results in thickening of the stem (radial growth). However, thickening growth can also occur during the primary growth phase through a process called **primary thickening growth** (PTG). PTG is subtle in most eudicots but plays a major role in USO development of aquatic eudicots and monocots [6]. PTG is responsible for the widening of stems in bulbous taxa to accommodate storage leaves and for thickening growth in rhizomes (reviewed in the study by Rudall et al. [7]). In monocots, PTG is carried out by the primary thickening meristem (PTM), which is continuous with the shoot apical meristem (Figure 2a, b; see the study by de Menezes et al. [8] for a different interpretation). While little is known about the molecular mechanism underlying PTM initiation in USOs of monocots, investigation of primary thickening in the photosynthetic shoots of Moso bamboo (*Phyllostachys edulis*) have implicated the ethylene and ABA pathways [9]. Further study of the molecular mechanism of PTG in USOs is necessary to understand USO thickening in diverse taxa.

Secondary thickening growth

Secondary thickening growth (STG) plays an important role in USO thickening in eudicots. In eudicots, STG occurs through lateral meristems such as the vascular cambium and cork cambium. While limited STG occurs in *Arabidopsis thaliana*, the molecular mechanisms underlying the initiation and proliferation of the vascular cambium have been well characterized in this model system (reviewed in studies by Ragni and Greb [10] and Turley and Etchells [11]). In *Arabidopsis*, the homeobox transcription factors WOX4 and WOX14 and the receptor-like kinase PHLOEM INTERCALATED WITH XYLEM (PXY) act downstream of auxin to promote vascular cambium formation and proliferation [10,12,13]. The *Arabidopsis* vascular cambium is highly informative for USO development; in a gene regulatory network analysis of USO development in carrot, cassava, and radish, roughly one third of genes upregulated in the *Arabidopsis* root cambium were also differentially expressed in the USOs [14]. Similarly, *WOX4* is upregulated in storage roots compared to fibrous roots of sweet potato and several other Convolvulaceae species [15]. In sweet potato storage roots, the MADS-box protein SRD1 promotes proliferation of metaxylem and cambium cells, leading to auxin-dependent thickening growth of storage roots [16]. In the swollen hypocotyl and taproot of radish, RsWOX4 directly upregulates *RsCLE22a* expression and silencing of *RsCLE22a* increases vascular cambium activity [17]. Taken together, evidence from multiple plant systems with root tuber or swollen hypocotyl USOs suggests that auxin and the WOX-CLE system (including the CLE signal peptides, their receptors, and their target homeodomain-containing WOX transcription factors) play a widespread role in proliferation of the vascular cambium [18].

In USOs derived from proliferation of the vascular cambium, xylem parenchyma are usually the major storage cells (Figure 2c, d). Little is known of the genetic differentiation pathway of xylem parenchyma compared to other xylem derived cells, although HD-ZIP III genes play an important role in *Arabidopsis* xylem differentiation [19,20]. In cassava storage roots, the HD-ZIP III gene *MeC3HDZ1* is highly expressed in the cambium and xylem and binds promoters of other storage root-expressed genes *in vitro* [21]. Transcriptome evidence from cassava suggests that KNOX/BEL genes may also play an important role in xylem parenchyma function [22]. Cassava fibrous roots and storage roots appear to differ in xylem parenchyma production early in development, suggesting that this cell type is critical for

Figure 1

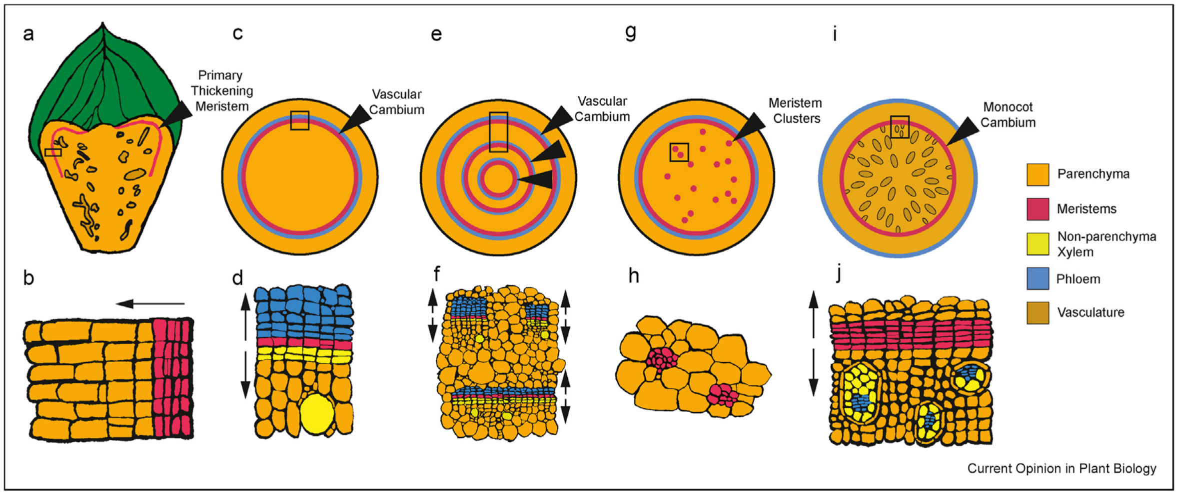


Conceptual model of USO development. **(a)** In potato, environmental signals such as photoperiod and temperature influence the expression of the key regulator SP6A in leaves (reviewed in the study by Zeirer et al. [25]). The SP6A protein travels from leaves to underground stems to induce stem tuber development and to promote thickening. Many known aspects of potato tuber development are not shown. **(b)** In cassava, a mobile protein such as a member of the PEBP family may respond to environmental signals and move to the roots to induce root tuber development and promote thickening growth. However, no functional evidence for such a mobile protein in the formation of storage roots has been obtained, so the key environmental signals and molecular mechanisms in cassava storage tissue development are still unknown.

storage root formation [23]. Further study of xylem parenchyma differentiation in general, as well STG in USO forming taxa, will improve our understanding of the mechanisms of USO development.

While the vascular cambium plays an important role in STG of many plants, others achieve STG via other diverse mechanisms. In the swollen hypocotyl and taproot of beet, additional, anomalous cambia form outside of the first, coinciding with increasing cytokinin and auxin levels (Figure 2e, f) [24]. In potato, cell division occurs mainly in the perimedullary zone of the pith. The origin of this pith is from procambium-derived meristematic centers, distinct from the vascular cambium (Figure 2g, h; reviewed in the study by Zeirer et al. [25]). Although most growth in a developing potato tuber occurs in the perimedullary zone, to our knowledge no studies test for anatomical changes in this region in transgenic plants. Some monocots also exhibit STG via a secondary thickening meristem (STM: also

Figure 2



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Diverse origins of thickening in underground storage organs. **(a, b)** Primary thickening as it occurs in onion (*Allium cepa*). (a) Schematic of a longitudinal section through a young onion stem. The primary thickening meristem (PTM) is continuous with the shoot apical meristem. (b) Zoom in of black rectangle in (a). **(c, d)** Secondary thickening in tuberous roots exemplified by cassava (*Manihot esculenta*). (c) Schematic of a cross section through tuberous root. (d) Zoom in of black rectangle in (c). The bifacial vascular cambium produces phloem centrifugally and xylem, mostly xylem parenchyma, centripetally. **(e, f)** Secondary thickening in swollen hypocotyl exemplified by beet (*Beta vulgaris*). (e) Schematic of a cross section through swollen hypocotyl main tissue types. **(**f) Zoom in of black rectangle in (e). Beets contain approximately concentric circles of anomalous cambium that arise from phloem and pericycle. **(g, h)** Secondary thickening in stem tuber as exemplified by potato (*Solanum tuberosum*). (g) Cross section of stem tuber. Meristematic clusters are randomly dispersed through the perimedullary zone. (h) Zoom in of black rectangle in (g). Meristem clusters contribute the large amount of parenchyma. **(i, j)** Secondary thickening in monocot rhizome exemplified by *Patersonia occidentalis*. (i) Schematic of cross section showing main tissue types in rhizome. (j) Zoom in of black rectangle in (i). Monocot cambium produces secondary vascular bundles and parenchyma centripetally and parenchyma cells centrifugally. The PTM and the monocot cambium are depicted as defuse, but see the study by de Menezes et al. [8] for alternative explanation. Arrow heads in (a, c, e, g, i) point to distinct meristems in each taxon; arrows in (b, d, f, h, j) indicate plane of divisions. The colors in the right panel indicate the important tissues during the origin of thickening in underground storage organs. Vasculature indicates the xylem + phloem tissues together in (a, i). The xylem derivatives (those immediately adjacent to the vascular cambium) and the mature non-parenchymatous tissues are colored yellow.

known as the monocot cambium, distinct from the vascular cambium), which underlies STG in some USO-forming Asparagales (Figure 2i, j, reviewed in the study by Tribble et al. [5]). To our knowledge, no studies characterize the molecular basis of the STM in USOs. However, expression of WOX4-like genes and PXY-like genes in the aboveground STMs of *Yucca* and *Cordyline* [26] suggests that some molecular mechanisms of the monocot cambium are similar to STG by the vascular cambium and likewise may underlie USO development. Broadly, the diverse mechanisms of STG that give rise to storage parenchyma require further molecular study.

Thickening through cell expansion

Following cell proliferation, cells in USOs must also expand to accommodate starches or other storage carbohydrates, which accumulate during USO development [27]. In beet, brassinosteroid signaling is necessary for parenchyma cell enlargement within the hypocotyl and dramatically contributes to the overall diameter of the swollen hypocotyl [28,29]. Transcriptomic studies indicate that lignin synthesis genes are underrepresented and expansin encoding genes (proteins that loosen cell walls) are upregulated in diverse eudicot USOs compared to non-modified organs [15,30–36], likely facilitating cell expansion to accommodate non-structural carbohydrates and lateral growth through cell expansion. In the root tubers of monocot *Bomarea* this pattern holds for lignin but expansins are instead underexpressed [37]. This suggests that the role of expansins in USO cell expansion may be lineage specific while reduced lignin is ubiquitous.

Mechanisms of USO induction by environmental signals

Competence to induce USO development

Before they are competent to begin USO thickening growth in response to environmental signals, some plants proceed through early developmental stages or produce specialized morphological structures. Many plants must transition through fixed ontogenetic stages corresponding to the age of the plant (sometimes termed vegetative phase change), a process that is not well-characterized in USO-forming taxa [25,38]. Some plants also produce specialized structures that bear USOs, which develop before USO thickening growth. For example, before normal tuberization in potato, the plant produces underground shoots known as stolons (but see the study by Herben et al. [39], where stolons are defined as an aboveground structure). The stolon undergoes elongation underground and develops a hook at the apex before thickening into a tuber [40,41]. The development of underground stems which give rise to stem tubers and the rhizomes found in many perennial taxa is not well understood [42], even though stolon elongation is an important step preceding tuber development. Interestingly, RNAi-knockdown of *BRANCHED1b* in potato allows plants to bypass the requirement for underground stolon development before tuberization; these plants produce aerial tubers directly from axillary buds [43]. Although tuber development occurs without stolons in this experimental case, stolon elongation is an important precursor to underground tuber development in wild-type potato. Once the stolons develop, short-day treatment induces a simultaneous end of elongation and beginning of tuberization, with a corresponding decrease in GA and increase in ABA [44,45]. Thus, proceeding through prior ontogenetic stages allows environmental factors such as photoperiod (below) to induce USO development.

Photoperiod signaling mediated by PEBP family proteins

Photoperiod cues induce USO development in diverse taxa through members of the phosphatidylethanolamine-binding protein (PEBP) gene family, which includes *FLOWERING LOCUS T PROTEIN* (*FT*) and other flowering regulators (e.g., potato [46], onion [47], Asiatic hybrid lily [48], *Bomarea multiflora* [37], reviewed in the studies by Khosa et al. [38] and Navarro and Prat [49]). The co-option of this gene family among various taxa and USO types reflects widespread coordination of below-ground nutrient storage with seasonal cues and flowering [38]. For example, differential regulation of the *FT* homolog *SELF PRUNING 6A* (*StSP6A*) by CYCLING DOF FACTOR 1 (StCDF1) underlies genetic variation in the day length required for USO development. Equatorial potato genotypes require short days to tuberize, but a truncated allele of the *StCDF1* gene allows some genotypes to tuberize in long days, enabling potato cultivation at high latitudes. The protein encoded by this truncated *StCDF1* allele is not degraded under long days and upregulates *StCONSTANS-LIKE1* (*StCOL1*), leading to lower levels of the tuberization-repressing FT-like gene *StSP5G* and higher levels of tuberization-promoting *StSP6A*, and as a consequence, tuber induction [50]. Similarly, onions are subject to photoperiod regulation through *AcFT1*, which promotes bulbing, and *AcFT4*, which inhibits bulbing by repressing *AcFT1* expression [47]. Thus, FT-like genes regulate USO formation in both monocots and eudicots and in a variety of USO types.

After *StSP6A* is upregulated in response to short days, it influences potato tuber development through multiple molecular mechanisms. StSP6A forms various protein complexes that promote tuber development, such as the tuberigen activation complex (TAC), composed of StSP6A, 14-3-3 proteins, and the transcription factor and FD homolog StFDL1, and the alternative TAC, which contains the ABA pathway transcription factor ABA INSENSITIVE 5-like 1 (StABI5-like 1) instead of StFDL1 [51,52]. The TAC regulates the AP1-like gene *StMADS1* which contributes to tuberization [53]. In addition to functioning as part of transcriptional regulatory complexes, StSP6A physically interacts with the sucrose efflux transporter StSWEET11 to promote potato tuber formation, supporting crosstalk between sucrose source-sink partitioning and photoperiodic pathways [54]. Thus, StSP6A regulates tuberization in potato by multiple molecular mechanisms. Further work to characterize the mechanisms by which FT homologs function in systems other than potato will improve our understanding of deep homology and shared regulatory pathways among different USO-forming taxa and types of USOs.

Temperature and drought regulation

Heat and drought stress also influence USO development. In potato, overexpression of *StSP6A* improves potato tuber yield under heat stress, suggesting that downregulation of *StSP6A* under heat stress mediates reduced tuber production [55]. The circadian clock gene *TIMING OF CAB EXPRESSION 1* (*StTOC1*) represses *StSP6A* transcription under heat stress, likely by preventing *StSP6A* autoactivation [56]. The microRNA *SES* is upregulated under heat conditions and represses *StSP6A* post-transcriptionally [57]. Comparisons of *StSP6A* and *SES* expression at different temperatures and developmental stages revealed that post-transcriptional regulation by *SES* plays a major role in *StSP6A* mRNA levels early in tuberization, while transcriptional regulation of *StSP6A* influences mRNA levels at later stages [58]. In chrysanthemum (*Chrysanthemum morifolium*), drought stress leads to a reduced number of rhizomes, likely by repressing the DEAD-box RNA helicase *CmRH56*, which in turn leads to GA degradation [59]. Given that *CmRH56* knock-down does not eliminate rhizome production entirely, it is likely that other regulators influence rhizome number in this system. Neither *SES* nor *CmRH56* have been implicated in USO developmental regulation in response to heat and drought in other systems. It is unclear whether temperature and drought regulation of USO development tends to proceed through taxon-specific mechanisms, or if further study of the mechanisms of USO regulation by heat, drought, and other factors would reveal shared pathways among taxa and USO types.

Conclusion

The above sections highlight the disparity in scientific understanding of molecular mechanisms underlying USO development. While certain crop systems (namely potato, Figure 1a) are well characterized for some stages of USO development (induction and expansion, less so for initiation of stem elongation), we still lack a basic understanding of molecular and developmental mechanisms across most USO types and plant lineages (Figure 1b). Moreover, we have not characterized the role of known molecular players in USO development in determining USO anatomy and morphology. We propose five particularly exciting avenues for future research on USO development:

1. Characterize the molecular and morphological basis of USO development in root tubers, bulbs, and other understudied USO types.
2. Investigate the morphological and genetic basis of thickening growth in eudicot USOs.
3. Determine the effects of manipulating known molecular players in potato development on tuber anatomy, particularly at the perimedullary zone, to integrate molecular knowledge with anatomical changes.
4. Investigate the molecular mechanisms of cell proliferation from two understudied lateral meristems, the primary thickening meristem and the monocot cambium.
5. Test for deep homology among USO types and taxa by studying orthologous genes in multiple taxa.

Filling these knowledge gaps requires employing genomic and transgenic approaches to test candidate genes in USO-forming systems other than potato. Although transformation in non-model systems can pose a challenge, several USO-forming taxa can be stably transformed through floral-dip methods and could be developed as new models for USO development (e.g. rhizomes in monkeyflower [60,61]; swollen hypocotyl and taproot in radish [62]). Important USO-forming crop taxa such as sweet potato, onion, and cassava could also be improved for USO developmental research with sufficient transformation protocols and genomic resources (e.g. the study by Zeirer et al. [63]). Finally, the wild relatives of crop taxa (such as *Oryza longistaminata*, a rhizomatous relative of cultivated rice) can be developed as models for USO research with tangible benefits for crop development [64,65]. Such studies should be accompanied by detailed morphological and anatomical characterization of transgenic lines to thoroughly describe the modifications of plant tissues through development (e.g. in the studies by Noh et al. [16] and Dong et al. [17]).

Despite the morphological and phylogenetic diversity of USO-forming plants, some molecular players such as PEBP family genes are frequently involved in USO regulation. This is even true of distantly related taxa such as potato and onion, a monocot and a dicot that undergo contrasting morphological mechanisms of thickening growth to produce a stem tuber and a bulb, respectively. Beyond PEBP family genes, it is unclear whether molecular mechanisms are relatively organ- and taxon-specific, are shared by similar USO types, or are shared by closely related taxa. By uncovering molecular mechanisms of USO development in diverse plant systems, we can determine the extent to which these mechanisms are shared among different USO types and diverse taxa. Since USOs serve as a source of protected buds and nutrients, understanding USO development broadly is a critical step toward understanding how plants survive harsh conditions. This knowledge has implications for the repeatability of growth form evolution and the nature of convergence at macroevolutionary scales.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

No data was used for the research described in the article.

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