Turning a Wild Plant into a Model – *A Déjà vu* Story

Daniel Ioan PACURAR [1), 2)]

[1)]*Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90133 Umeå, Sweden*
[2)]*University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, Romania; Daniel.Pacurar@genfys.slu.se*

Abstract

In the past two decades we have witnessed how a useless wild weed has been transformed from an anonymous into a model plant, probably the most widely "cultivated" plant species. The process has been rather slowly in the beginning, very laborious on the way, extremely expensive and time consuming, but the outcome is priceless – the knowledge that is most likely to frame and fill the blueprint of the first artificial plant, as system biology promises. The plant species is *Arabidopsis thaliana* and the "growers" are highly qualified researchers worldwide. This review introduces a new anonymous – *Brachypodium distachyon* – that raised big hopes for addressing specific problems of fundamental and practical biology in temperate cereals and forage grasses, and is rapidly becoming a "sweetheart" for the researchers working with these crops, and not only.

Keywords: Brachypodium distachyon, Arabidopsis thaliana, Oryza sativa, model plant species

Introduction

Since the onset of humanity we have domesticated wild plants to provide us with food, fibres and oils; we selected them for medical purposes, or not less important as ornamental companions of our daily life. The fates of civilizations depended on complex interactions between social, environmental, and biological factors – one of the latter being the nature of the major crop(s) being cultivated by each society (Murphy, 2007). Over time we have improved and augmented the desired characteristics of what we all know as crops, technical, medicinal or ornamental plants. Recently, the domestication of switchgrass (*Panicum virgatum*) for the production of biofuel added a new group – of energy crops. The domestication of the above-mentioned plants had a clear, straightforward purpose – of providing the developing society with resources. The resources generate growth, and growth lays the foundation for more and sophisticated knowledge that fuels back the society's development.

An extremely important group of plant species that has equally provided us with resources and knowledge is the group of so called model plants. In this group are included economically important crop plants like rice (*Oryza sativa*), corn (*Zea mays*), wheat (*Triticum aestivum*), garden pea (*Pisum sativum*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), tobacco (*Nicotiana tabaccum*), *Medicago truncatula* and *Lotus japonicus* (Păcurar, 2009), but also species that have been domesticated and served exclusively as models for plant genetics and development. The

last subgroup has two representatives: the classical model *Arabidopsis thaliana* and the newcomer *Brachypodium distachyon* that has been promoted and is being developed as a model for temperate cereals and forage grasses.

Not surprisingly, Arabidopsis was mentioned back in 1777 by the British botanist William Curtis as a plant of no particular virtue or uses (Mahalakshmi and Ortiz, 2001) and remained so until it was chosen to be the first model plant. Today *Arabidopsis thaliana* is maybe the most described plant in the world, with a large collection of characterized mutations and transgenic plants available, through which most aspects of plant growth and development have been disrupted. Furthermore, the efforts and productivity of the Arabidopsis research community has led to sequencing of the Arabidopsis genome in 2000 (The Arabidopsis Genome Initiative, 2000), thus Arabidopsis being the first plant species whose genome sequence has been fully completed and is publicly available. The community is now engaged in an international collaborative effort to determine the function of the plant's ~26.000 estimated genes (Chory *et al.,* 2000).

The discoveries made during the past 20 years by using Arabidopsis as a model plant have fundamentally changed plant biology (Somerville, 2000) and predictions are that Arabidopsis will continue to be of central importance in plant biology for the near future (Somerville and Koornneef, 2002). However, as a dicot species, Arabidopsis does not share with grass crops many biological features important from an agricultural perspective, therefore it cannot be
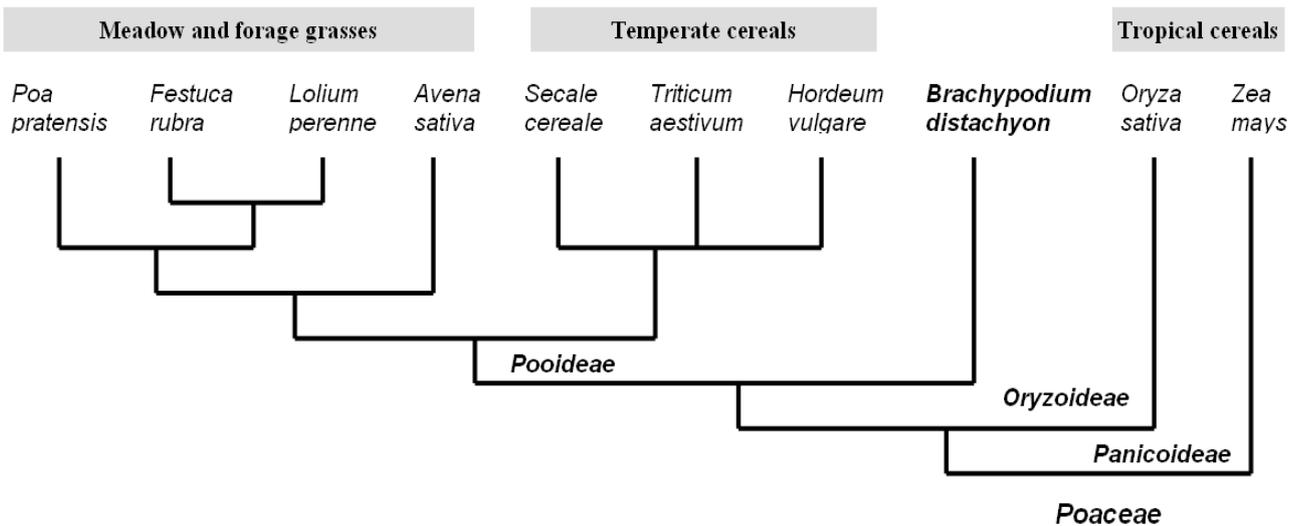
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Fig. 1. Phylogenetic relationship of *B. distachyo*n to other *Poaceae* (figure modified from Draper *et al.*, 2001)

considered an ideal model for grasses (Devos *et al.,* 1999; Tikhonov *et al.,* 1999; Keller and Feuillet, 2000).

With its international status as a staple food source and many years of intensive breeding, rice (*Oryza sativa*), with its compact genome, seems to be a better alternative. Rice has been promoted as a model for cereal genomics (Havuk-kala, 1996; Goff, 1999), and as for Arabidopsis, consider-able international effort has been put in developing it as a complete model, leading to the completion of its genomic sequence in 2002 (Yu *et al.,* 2002; Goff *et al.,* 2002). De-spite this, the value of rice as a model for the temperate cereals and forage grasses may be questionable (Draper *et al.,* 2001). One of the disadvantages using *O. sativa* as model plant for grasses is that it belongs to the *Oryzoideae* subfamily which is phylogenetically distant related to the *Pooideae* subfamily (Figure 1) comprising temperate grass-es like *Festuca rubra* and *Lolium perenne* as well as impor-tant cereals as *T. aestivum* and *H. vulgare* (Christiansen *et al.,* 2005). Many temperate grasses need vernalisation and long-day conditions in order to induce flowering. *O. sativa* deviates from this group of grasses being a short-day plant without vernalisation requirement. In addition, rice is a relatively large, outbreeding plant with a comparably long life cycle and demanding growth requirements, thus im-posing some practical limitations. Moreover, many traits that are relevant to be studied in temperate crops (e.g. re-sistance to specific types of pathogens, freezing tolerance, vernalisation, perenniality or post-harvest biochemistry of silage) are not exhibited or rarely studied in rice (Draper *et al.,* 2001).

These limitations of rice to provide a robust model sys-tem for the temperate crops opened the challenge to evalu-ate the complementary opportunities offered by other spe-cies in the *Pooideae*, in order to identify a grass species that has the potential to be developed into a model representa-tive for all grass crops. The grass genus of *Brachypodium* has been investigated (Robertson, 1981; Catalan *et al.*, 1995;

Khan and Stace, 1999; Draper *et al.,* 2001; Hasterok *et al.,* 2004) and its only true annual species, *Brachypodium dis-tachyon* (L.) Beauv. has been proposed as a model plant for temperate forage grasses (Draper *et al.,* 2001).

The next part of this review will focus on *B. distachyon* description and characterisation, with emphasis on the ad-vances made in the process of raising it to the stage of a model.

Brachypodium distachyon identity card: phylogeny and botanical characterization

The genus *Brachypodium* (from the Greek *brachys* "short" and *podion* "a little foot", [Watson and Dallwitz, 1992]) comprises relatively few temperate grass species, yet with a wide distribution (Figure 2), two features that according to Tateoka (1968) indicate a very ancient ori-gin. He suggested that the genus has become well diver-sified by the mid Tertiary and estimated a total of 14 to 18 species. This enigmatic genus whose phylogenetic rela-tions, generic definition and specific habitat delimitations have always been the subject of debate was first described by Palisot de Beauvois in 1812 with 22 species (Khan and Stace, 1999). Only five of these species are included today in the genus *Brachypodium*. Schippmann, (1991) identi-fied seven species in Europe and one endemic species in the Canary Islands. Elsewhere the presence and distribu-tion of the genus need to be revised: 2-7 species occur in southern Africa, 1-6 species occur in Central America and an estimated number of maybe 17 occur in eastern Asia (Khan and Stace, 1999). In Romania, *Brachypodium dis-tachyon* can be found on sands, grassland and cultivated lands from Tulcea County and Danube's Delta at Sf. Ghe-orghe (Prodan and Buia, 1966), with its perennial relative *B. sylvaticum* having a wider distribution. The genus *Brac-hypodium* is in need of a world monograph and Catalan

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Fig. 2. Geographic distribution of the genus *Brachypodium* (in green) (Garvin *et al.*, 2008)

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* – Grass family
Sub-family	*Pooideae*
Tribe	*Brachypodiae*
Genus	*Brachypodium* **P. Beauv.** – False brome
Species	*Brachypodium distachyon* (**L.**) **P. Beauv.** – Purple false brome

Fig. 3. Systematic classification of the species *B. distachyon*

et al., (1995) assumed that it probably contains 12 to 15 species.

The phylogenetic status of the genus has been controversial over the years, but recent studies based upon cytological, anatomical and physiological characterisation places *Brachypodium* into its own tribe *Brachypodieae*, in the family of *Poaceae* (Hasterok *et al.*, 2004). In support of this taxonomic classification, molecular phylogenetic investigations have consistently placed the *Brachypodieae* into the sub-family *Pooideae* (Figure 3), which includes the most important temperate cereals (Shi *et al.,* 1993; Catalan *et al.,* 1995, Catalan *et al.,* 1997; Catalan and Olmstead, 2000). Furthermore, it appears that the genus *Brachypodium* is a distinct clade, which diverged soon after the division of the *Pooideae* from the *Oryzoidae* (Figure 1) and is therefore more closely related to the temperate cereals and grasses than is rice (Draper *et al.,* 2001).

Fig. 4. *B. distachyon*

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The genus *Brachypodium* include annuals to strongly rhizomatous perennials, strictly inbreeding to highly self-incompatible species (Khan and Stace, 1999). Brachypodium distachyon (L.) Beauv. or purple false brome [synonyms: Bromus distachyos L., Trachynia distachya (L.)] (Figure 4) is the only true annual species of the genus. Plants are loosely tufted, bright green or glaucous, 20-60 cm high (in general, the diploid accessions are lowest). The culms are geniculate or stiffly erect, internodes glabrous, nodes conspicuously pubescent. The leaves are cauline with blades 10-40 cm long, 2-5 mm wide, flat, glaucous, sparsely hairy, veins unequally prominent, margins thickened, sparsely hairy, sheaths usually glabrous; ligules 0.5-2 mm, pubescent. The inflorescences 2-7 cm, with 1-7 usually overlapping, spikelets laterally compressed, with 7-15 florets. The caryopses are 5.7-7.8 mm, ellipsoid with hairy apices.

Genetics of B. distachyon

Brachypodium differs from all the other genera of the *Pooideae* in its small chromosomes (Robertson, 1981; Khan, 1984). The species of the genus shows also a variable chromosome base number (x = 5, 7, 8 or 9), compared with other species in the *Pooideae* that have large chromosomes and a base number of 7 (Shi *et al.,* 1993). The ploidy level range from diploid to octoploid even among the European species alone (Khan and Stace, 1999).

The earliest reports for *B. distachyon* were 2n = 30 (Fernandes and Queiros, 1968). Roux (1957) and later Kozuharov *et al.* (1974) provided support for x = 5 as the true base number of *B. distachyon* after both reported chromosome counts of 2n = 10. This suggested that *B. distachyon* has evolved as a polyploid series (2x = 10, 4x = 20 and 6x = 30), based on a 2n = 10 diploid, with the hexaploid 2n = 30 the most frequent cytotype (Draper *et al.,* 2001). Further support for such an interpretation was provided by the report of the tetraploid 2n = 20, made by Talavera, (1978). However, current data shows that the 20 chromosome cytotype differs substantially from the others, suggesting that the genetics in the polyploids is more complex than it was thought (Hasterok *et al.,* 2004; Garvin *et al.,* 2008).

Nuclear genome size in the diploid *B. distachyon* was determined by flow cytometry to be in the range between 172 Mbp/1C and 355 Mbp/1C, depending of the accession (Draper *et al.,* 2001; Bennett and Leitch, 2005), thus the second value is considered closer to reality. This is, however, the smallest known genome size in grasses, being intermediate between those of Arabidopsis (125 Mbp/1C) and rice (490 Mbp/1C) (Ozdemir *et al.,* 2008), and containing less than 15% highly repeated DNA (Shi, 1991; Catalan *et al.,* 1995).

Therefore, *B. distachyon* has several attributes in relation to its phylogenetic position, and by virtue of its small genome that make it useful for e.g. evolutionary studies in the *Pooideae*, thus providing a good platform for the analysis of the much bigger genomes possessed by important temperate grass crops, whose genomic research is often hampered by large genome sizes and polyploidy, and whit whom Brachypodium is expected to exhibit a greater synteny than rice or sorghum sequenced genomes (Draper *et al.,* 2001; Garvin *et al.,* 2008).

Desirable attributes for a model plant

For a plant species, in order to be positively evaluated as a potential candidate to be developed as a model, it needs to fulfil several characteristics that are important from a practical and economical perspective. Common requirements for model plants are: short life cycle, small genome size, availability of diploid ecotypes, low repetitive DNA content, small adult size, large number of offspring, ease to handle and low cost of production. Established laboratory tools for structural and functional genomics strengthen a plant model system. These include efficient transformation protocols, gene tagging protocols and DNA micro array systems, a well-developed genetic map, EST databases and a complete genome sequence. Together with the favourable biological characteristics and the developed laboratory tools, financial and political attitudes play an important role for scientist's acceptance and establishment of a model plant.

As for *B. distachyon*, selected tetra- and hexaploid accessions have a seed to seed life cycle of down to 3½ to 4 months while the diploid accessions have a seed to seed life cycle of 4½ to 5 months due to the vernalisation requirement and a slightly slower growth. Diploid accessions with short generation times (down to 2 months) have been selected too and flowering of many accessions can be accelerated under long-day conditions, continuous light and by vernalisation. However, flowering may be induced in the diploids by long day conditions alone, without the need for vernalisation (Vogel *et al,* 2006a; Garvin *et al.,* 2008). Mature *B. distachyon* plants have a physical size of 20 to 60 cm depending on accession (Christiansen, 2004). This enables handling large set of plants in growth chambers or greenhouses. Table 1 illustrates how Arabidopsis fulfils all requirements to be a perfect model plant, but *B. distachyon* also scores highly, the only remaining unfavourable feature is the smaller seed yield compared with Arabidopsis. However, compared with rice, *B. distachyon* has a more rapid life cycle, smaller stature, and it is a self-fertile plant that means that F_1 seed can be collected without the need for hand pollination or the time-consuming bagging of flowering plants.

On the way to become a model

In less than a decade, and preponderantly in the last couple of years the growing interest in Brachypodium is reflected in the number of publications that slowly pave

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Tab. 1. Comparison of established model plants and *B. distachyon* (table modified from Draper *et al.*, 2001; Christiansen *et al.*, 2004; Păcurar, 2005)

	A. thaliana	*O. sativa*	*B. distachyon*
Botanical class	Dicotyledones	Monocotyledones	Monocotyledones
Plant family	*Crucifereae*	*Poaceae*	*Poaceae*
Height at maturity (cm)	15-20	~ 90 (var. indica)	20-60 (in general, the diploid accessions are lowest)
Life cycle (months)	~ 1½-2	~ 4 (var. indica)	2-5 (diploid accessions) ~ 3½-4 (tetra- or hexaploid accessions)
Planting density (plants m-2)	~ 300	8-10	~ 300
Seed yield plant -1	> 1.000	> 1.000	80-200
Seed yield m-2	> 300.000	> 8.000	24.000-60.000
Growth requirements	simple	relatively specialized	simple
Chromosome no.	2n = 10	2n = 24	2n =10, 2n =20, 2n = 30
Genome size (1C)	125 Mbp	441 Mbp	< 355 Mbp
Breeding strategy	self fertile	out breeder	self fertile
Transformation system	available	available	available

its way to the status of a model. Since the breakthrough paper of Draper et al. (2001) many research groups have directed their interest and resources in studying Brachypodium, and the achievements reported have confirmed and strengthened its potential as a model plant system. The International Brachypodium Initiative (IBI) founded in 2005 with the aim to promote and support communications and collaboration in the Brachypodium research community will enforce these efforts. And, as a crowning of these efforts, the Brachypodium genomic sequence is expected to be completed soon, together with a transcriptome sequencing project aiming to sequence nearly 200.000 expressed sequence tags (ESTs) from different tissues (Garvin *et al.,* 2008). Hitherto, the first sequencing project in Brachypodium has been successfully completed; its chloroplast genome sequence being recently published (Bortiri *et al.,* 2008). Analyzes of Brachypodium chloroplast genome sequence revealed that it contains 118 unique genes, 18 of which being duplicated in the Inverted Repeats (IR). This makes a total of 136 genes of known function. In addition, there are 9 predicted open reading frames (ORFs) and 3 tRNA pseudogenes. With the forthcoming nuclear genome sequence, functional genomics is about to begin in Brachypodium.

In the new world context of fossil fuel shortage and increasing energy needs, the availability of Brachypodium complete genome sequences is expected to urge the functional genomics investigations directed e.g. towards specific modification of the plant cell wall for cellulose ethanol conversion. Therefore, the development of Brachypodium as a model is of particular value in the areas of cell wall and biomass research, where dicots and monocots showed to be extremely different (Gomez *et al.,* 2008).

As previously mentioned, in order to fully exploit the potential of a plant model system, a set of molecular and genomic tools need to be established. To start with, it is imperatively to have genetically homogenous and homozygous stock seeds available to the research community. In this context a set of homozygous inbred lines was developed by single-seed descendent, and are available on request (Vogel *et al.,* 2006a; Garvin *et al.,* 2008). However, in order to capture more natural variation in the species, additional inbred lines need to be produced. This will facilitate functional genomic studies. Further, development of a genetic linkage map of *B. distachyon* (Bevan *et al.,* 2007) will provide the support for genome comparison with cereal and biofuel crops and for genetic mapping.

Part of the effort in developing Brachypodium as a model was driven by the hope that its small genome can serve as anchor for synteny comparison with its crop relatives. Some recent preliminary results (Hasterok *et al.,* 2006, Faris *et al.,* 2008) have confirmed this hypothesis, the genome of Brachypodium sharing conserved regions with other grasses. This important feature will help in the process of accessing genetic regions of interest in the much larger genomes of economically important grass crops and for evolutionary studies of the *Poaceae*.

One of the first prerequisites in developing a model plant system is the availability of an efficient transformation protocol (Păcurar *et al.,* 2008). Taking advantage of the available tissue culture and regeneration system previously described by Bablak *et al.* (1995), both, microprojectile bombardment and *Agrobacterium*-mediated transformation protocols have been developed for Brachypodium. However, since the average transformation efficiency reported in biolistic transformation method of up to 5.3%

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(Christiansen *et al.,* 2005) is not adequate for large-scale genomics projects, efforts have been conducted in developing more efficient *Agrobacterium*-mediated transformation protocols. Our recently published *Agrobacterium* - mediated transformation protocol describes a high throughput method for the diploid accession BDR018 (Păcurar *et al.,* 2008), with transformation efficiencies of up to 80%, which are the highest reported for graminaceous species. The highly efficient transformation protocol developed in our study is pivotal for developing Brachypodium into a widely accepted model, and opens the door for large-scale transgenic approaches in *B. distachyon.* Moreover, it can serve as a starting point in improving the transformation efficiencies for the reference genotype Bd21, which is being used in most of the genomic research and as a DNA and RNA source for sequencing (Garvin *et al.,* 2008).

Additional molecular tools and genomic resources are continuously developed for Brachypodium, including mutagenesis techniques (Engvild, 2005), bacterial artificial chromosome (BAC) libraries (Hasterok *et al.,* 2006), expressed sequence tags (ESTs) collections (Vogel *et al.,* 2006b), metabolite profiling and fingerprinting technologies (Beckmann *et al.,* 2008). Furthermore, development of bioinformatics tools, including a database, is necessary to facilitate exploitation of the forthcoming genomic sequence.

Several preliminary studies recently conducted in Brachypodium, addressing specific problems of plant-pathogen interactions (Draper *et al.,* 2001; Routledge *et al.,* 2004; Jenkins *et al.,* 2005; Parker *el al.,* 2008) or functional genomics (Jenkins *et al.,* 2005; Olsen *et al.,* 2006), reported promising results, thus confirming its potential as a model. A lot is to be done, yet, in the area of plant-pathogen interactions or in the analysis of genetic and biochemical events underlying grain filling and quality, which for a *Pooid* model is an extremely important trait. Preliminary studies on seed storage proteins have been reported (Khan, 1992; Laudencia-Chingcuanco and Vensel, 2008), but seed development in *B. distachyon* has yet to be extensively analyzed. Finally, other possible directions for functional genomics in Brachypodium can be envisaged including freezing tolerance, vernalization, repetitive injury tolerance, meristem dormancy mechanisms, perenniality, post-harvest biochemistry of silage and hay, mycorrhizae and sward ecology (Draper *et al.,* 2001). All these are important features to be studied in temperate forage grasses and many temperate cereals.

Based on current evidences, *B. distachyon* has great potential to be the monocot counterpart of Arabidopsis, whit whom shares similarity in many aspects of its biology such as genome size, chromosome number, height, planting density, breeding system and duration of life cycle. Nonetheless, *B. distachyon* can be developed as a complementary model to rice offering thus the possibility to study grass traits that are not well exhibited by rice. In addition, its requirement for a simpler logistic of handling large populations, as compared with rice, can make *B. distachyon* an attractive alternative where investigative resources, such as access to controlled growth environments, are limited (Draper *et al.,* 2001).

Will "Brachy" have the blessed fate of Arabidopsis? We will be able to answer this question in the near future. If yes, it is certain that the whole process will be much faster, mainly due to the achievements from its dicotyledonous counterpart *A. thaliana.*

Concluding remarks

Not very far in the future we will be able to name the minimum set of genes a plant need in order to germinate, grow, flower and set seeds. Plant breeders have traditionally addressed this question, interestingly, by an inverse approach – how many genes a plant doesn't need and still can survive? This paradox is explained by the fact that in breeding often "less is more": less functional genes = e.g. bigger fruits, more seeds, increased content of desirable metabolite, higher production. It is true that with this approach we lost some in resistance, for instance, but we gain a "green revolution" that made possible in the past six decades supplying food for an increasing world population. Now another green revolution is desperately needed in order to feed the world, to fuel our cars, to give us energy. And the plants are called to save once again the humankind. The past has shown that we can successfully use the knowledge from the model plants to address more specific questions in crop plants. In this context the established model plants like Arabidopsis, rice, poplar or *Brachypodium*, and the new ones to enter the field (Păcurar, 2009) will play an enormous role.

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