



宮崎大学
University of Miyazaki



草類遺伝資源育種学研究室
Laboratory of forage grass genetic resource and breeding

Toward a New Understanding of Zoysia Diversity: A Reassessment of Its Classification Based on Digital 3D Imaging and Genomic Data

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About me



Sorawich Pongpiyapaiboon

Education:

~ **2017** Kasetsart University Laboratory school (Satit Kaset)

2017-2021 University of Miyazaki, Faculty of Agriculture,
Department of Animal and Grassland science

2021-2023 University of Miyazaki, Graduate School of
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2023-March 2026 University of Miyazaki, Interdisciplinary
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Work:

April 2026~ Researcher at SDS Biotech KK., Japan

Zoysiagrass (*Zoysia*)

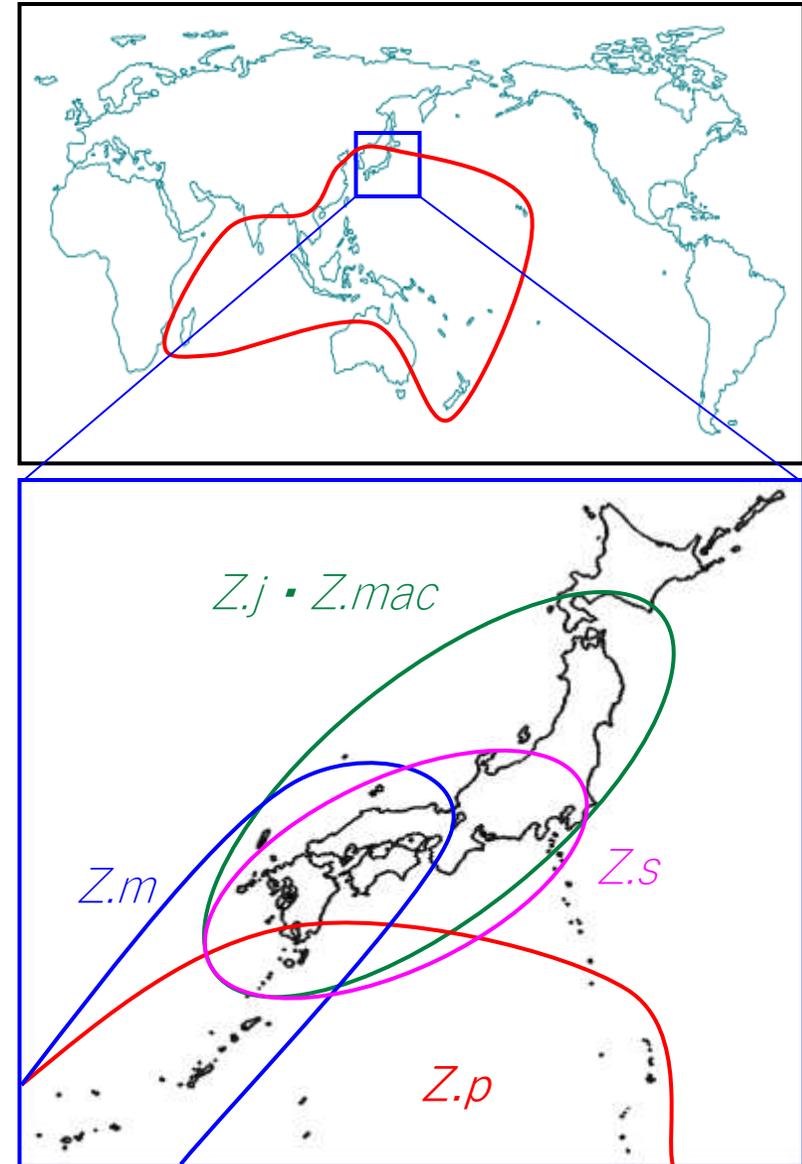
Genus *Zoysia*

The genus *Zoysia* is a warm-season turfgrass widely distributed across Asia, extending south to New Zealand, west to Madagascar, and east to the Hawaiian Islands.

Although numerous synonyms exist, the following five species are native to Japan:

- *Shiba* (*Z. japonica*)
- *Koushun-shiba* (*Z. matrella*)
- *Kourai-shiba* (*Z. pacifica* (*Z. tenuifolia*))
- *Oni-shiba* (*Z. macrostachya*)
- *Kooni-shiba* (*Z. sinica*)

Its chromosome number is $2n=40$, allowing interspecific hybridization, and hybrid cultivars have also been developed. It is used as lawn grass in gardens and as a forage species for livestock grazing. Zoysiagrass also have deep connection with cultural aspect of Japan



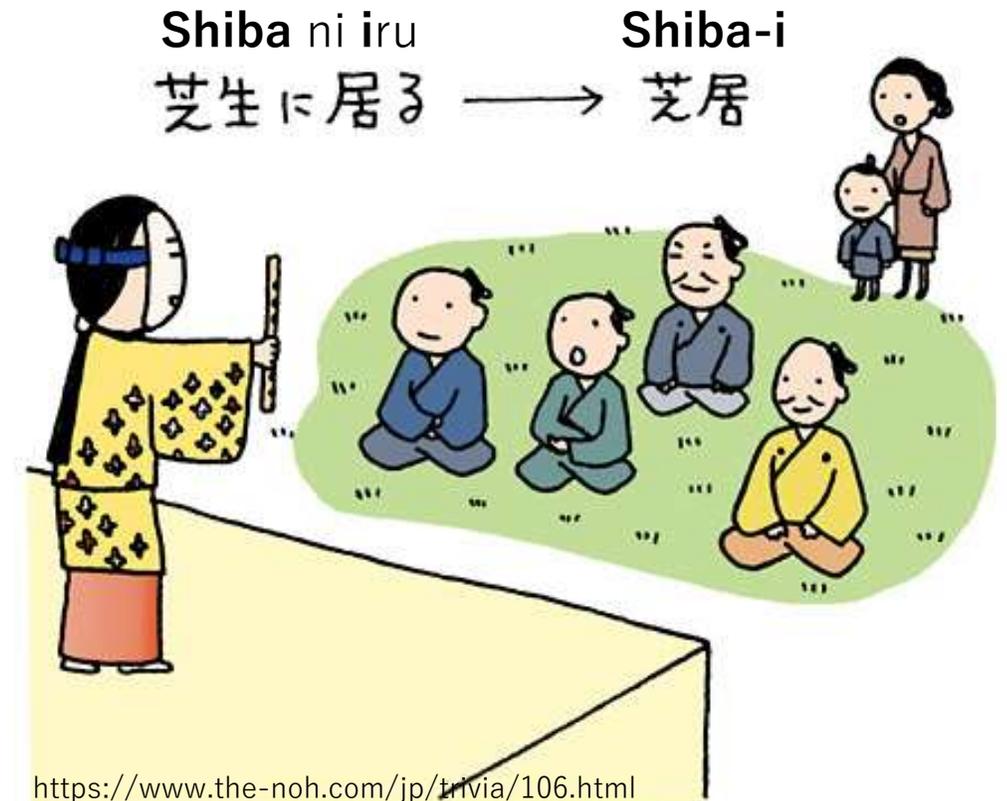
Word ‘Shibai’ and its connection to *Zoysia*

In Japan, zoysiagrass has been used since the Heian period (794~) as lawn grass in gardens and park. Also, as a forage grass for livestock grazing in mountainous and wild areas

In the Muromachi period (1336~), traditional performances and entertainments like ‘Noh’ and ‘Kagura’, were often held in the precincts of temples and shrines where people could easily gather.

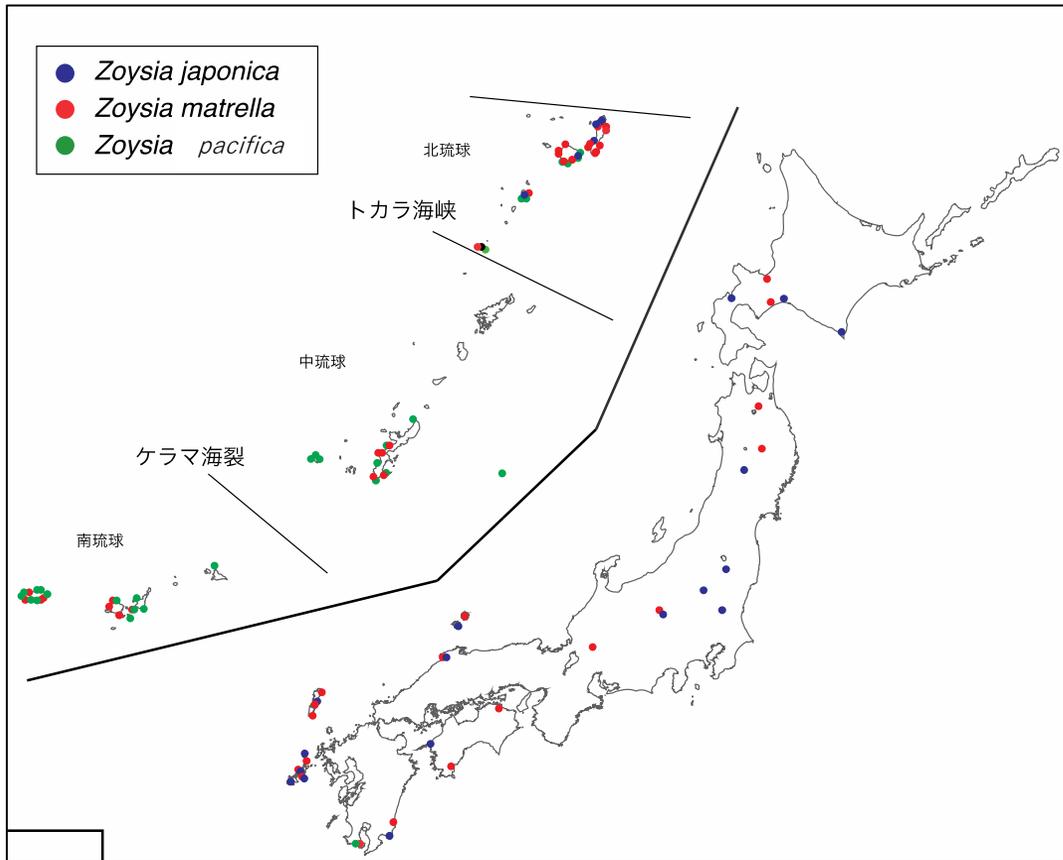
At that time, there were no fixed audience seats, so people sat on the grass to watch. It is said that the word **shibai** originated from this situation—literally from people “being (sitting) on the grass” (shiba = grass, i/iru = to be/sit).

Even now, the word ‘**Shibai**’ mean traditional play, show, theater, opera, and generally people still use and say ‘to watch shibai’



This story highlights **the deep cultural connection** between shiba (zoysiagrass) and Japanese society, extending beyond landscaping into historical and cultural practices.

Zoysiagrass in the wild



Confusion in identifying zoysiagrass

Poaceae family

Genus *Oryza*

Different species easy to identified

Genus *Zoysia*

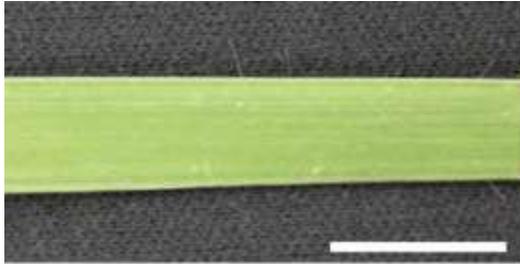
Different species hard to identified



Sanchez, Paul & Wing, Rod & Brar, Darshan. (2013).
The Wild Relative of Rice: Genomes and Genomics. 10.1007/978-1-4614-7903-1_2.

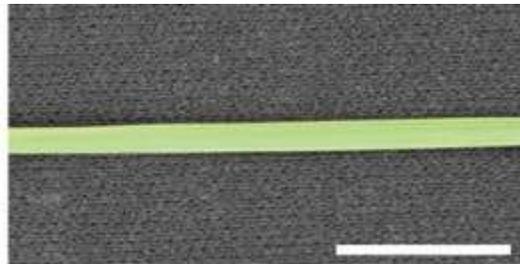
Characteristics of 3 main *Zoysia* species

• *Z. japonica*



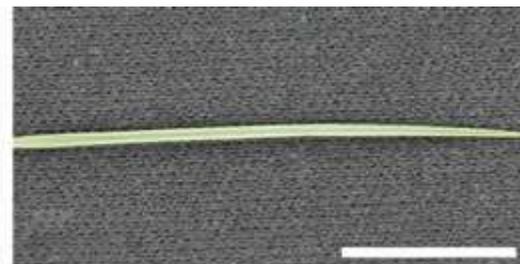
- Excellent growth potential
- Protective plants for **road embankments** and **pasture grass** species
- Most cold-tolerant within its genus
- **High dormancy**

• *Z. matrella*



- Narrow leaf width forms beautiful turf
- Suitable for **parks and stadiums**
- High salt tolerance, **moderate dormancy**

• *Z. pacifica*



- Forms compact, dense, beautiful turf
- Suitable for **low-traffic, small-scale areas**
- Slow growth (low maintenance)
- Highest salt tolerance and **low dormancy**

Flowers (spikelets) with pistils



Creeping stem of zoysiagrass



Comparison of *Zoysia* morphology

Parts	Traits	<i>Z. japonica</i>	<i>Z. matrella</i>	<i>Z. pacifica</i>	<i>Z. macrostachya</i>	<i>Z. sinica</i>
Plant	Plant length	15 cm ~	10~25 cm	10~20 cm	-	10-25 cm
	Leaf length	4.0 cm~	1.0~4.0 cm	2.5~5.0 cm	3.0~5.0 cm	3.0~7.0 cm
Leaf	Leaf structure	Triangular type / moderately broad type	Triangular type / moderately broad type	Linear type	Moderately broad type	Linear type
	Leaf width	2.0-5.0 mm	1.0-4.0 mm	1.0 mm以下	2.0-4.0 mm	2.0~3.0 mm
	Leaf cross-section	H-Type·V-Type	V-Type·U-Type	V-Type·R-Type	H-Type·V-Type	V-type or U-type
	Large vascular bundles	7~	~7	~3	~7	~7
	small vascular bundles	Around 30 (4~6 between each large vascular bundle)	Around 20 (3~4 between each large vascular bundle)	Around 6 (2~3 between each large vascular bundle)	-	-
	Leaf bud (vernation)	Spirally coiled about turns (large diameter)	Spirally coiled about 1.5~2 turns (medium diameter)	Folded in two (conduplicate) (small diameter)	Spirally coiled about 2~2.5 turns (large diameter)	Spirally coiled
	Runner	Stem length	10-20 cm	7-20 cm	7-20 cm	15-20 cm
Panicle length		3-5 cm	1-3 cm	1-3 cm	3-4 cm	3-5 cm
Panicle width		2-7 mm	2-4 mm	2-4 mm	6-8 mm	-
Flower	Spikelets length	3 mm	2.5-3.5 mm	2.5-3.5 mm	6-8 mm	4-5 mm
	Spikelets width	1.2-1.5 mm	0.5-0.8 mm	0.5-0.8 mm	1.8-2.2 mm	-
	Flowering period	May - July	May - October	September - November	May - September	May-June

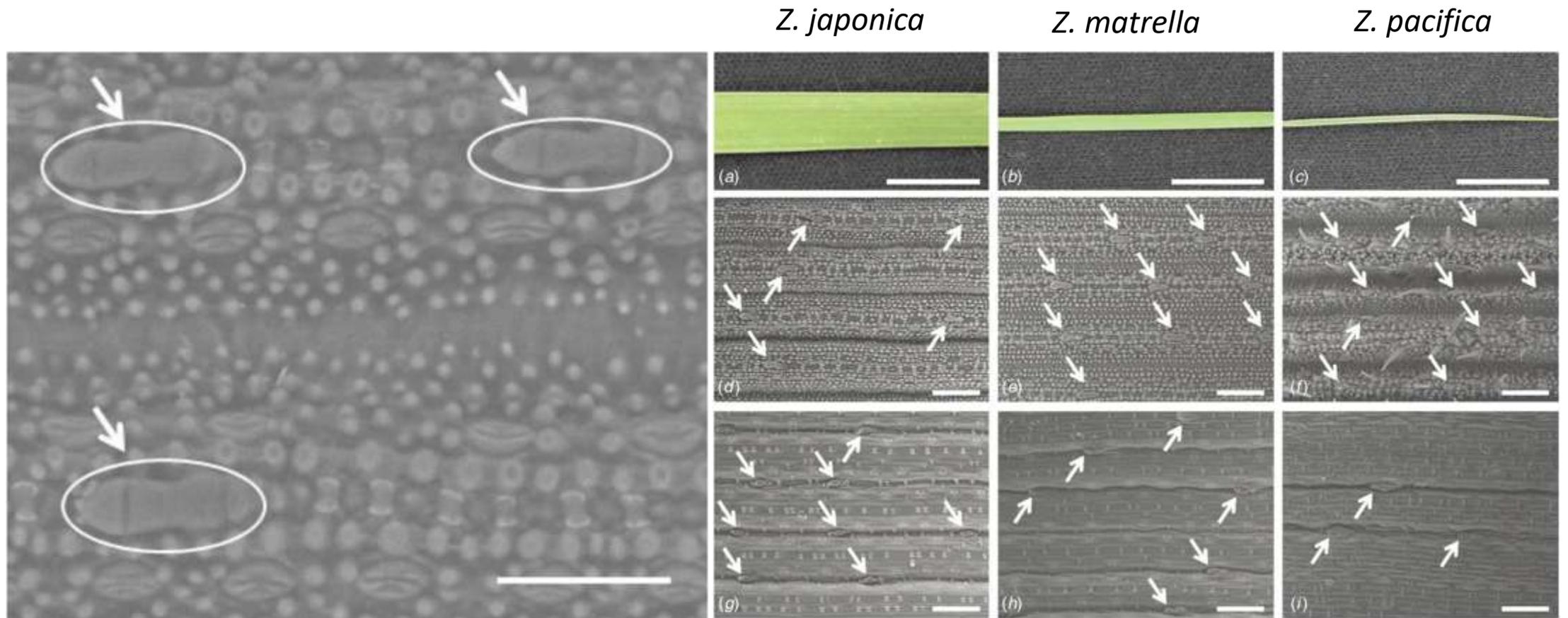
Zoysiagrass winter dormancy



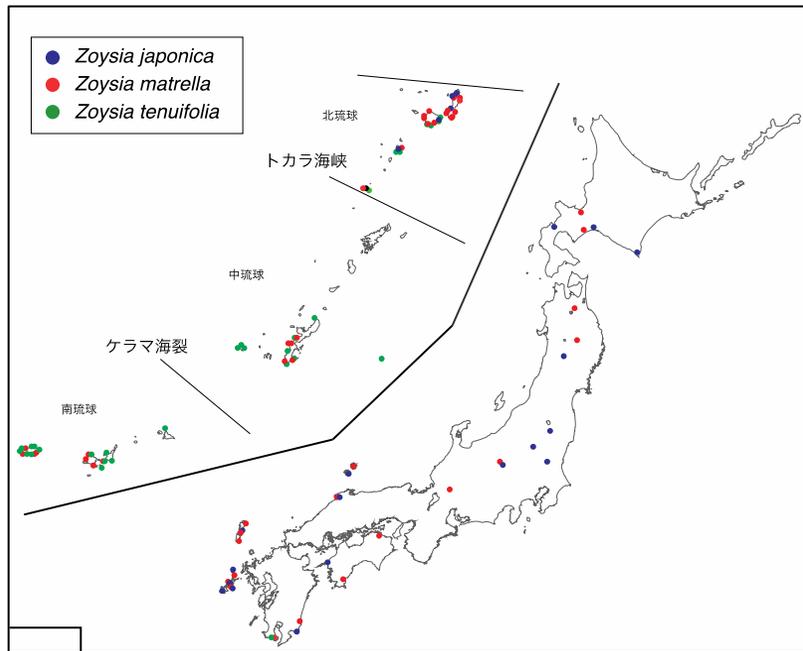
Arima CC. Hyogo prefecture, Japan
<http://www.arimacc.jp/report/post-6766>



Salt tolerance: Leaf blade salt gland

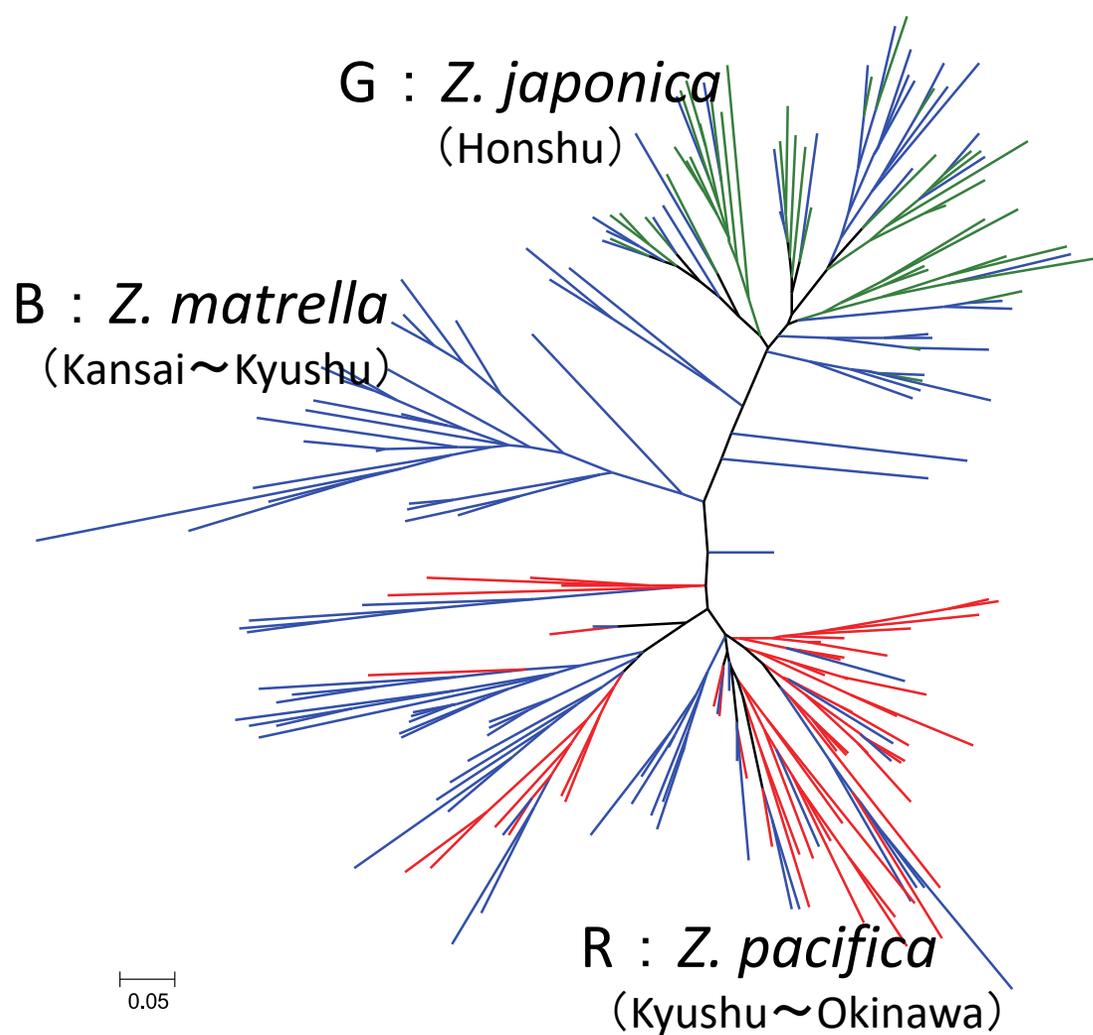


Zoysia genetic resource and breeding



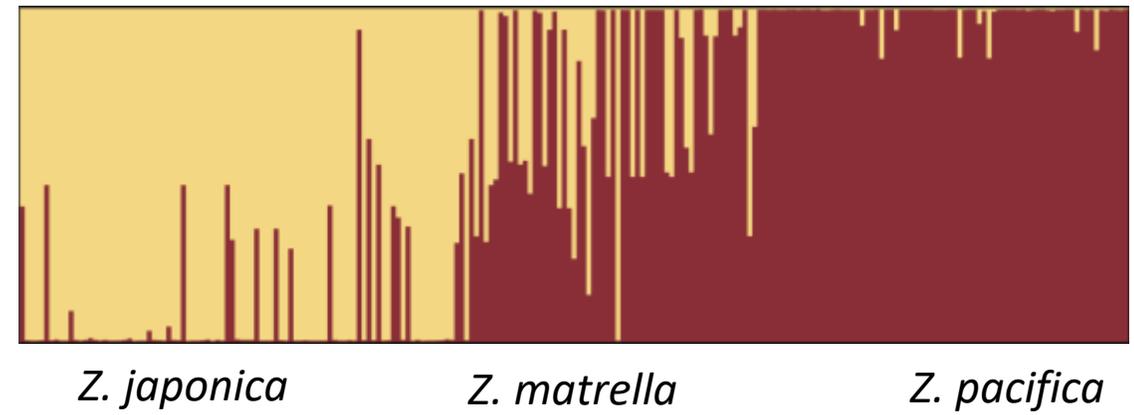
300+ wild accessions from Hokkaido to Kyushu and southern islands

Phylogenetic Analysis of the Genus *Zoysia*

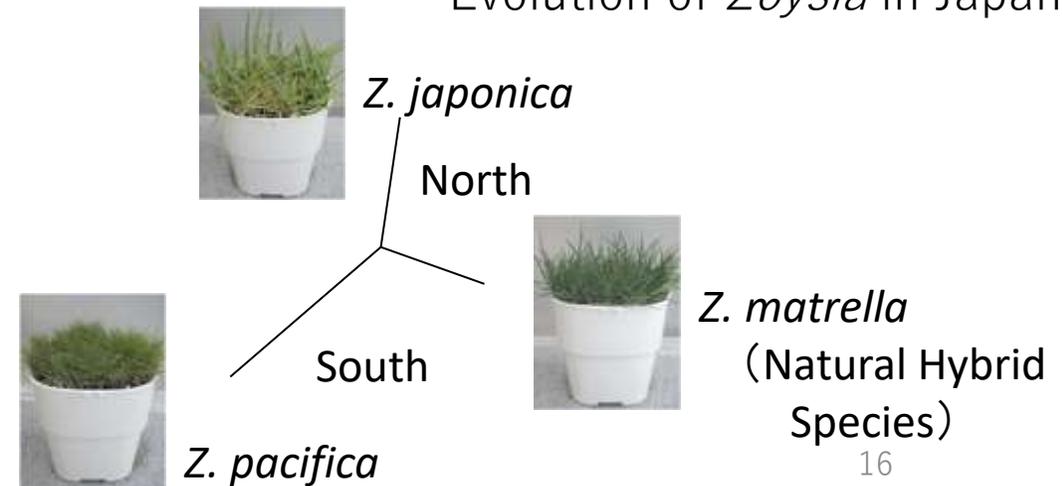


Tanaka et al., 2016

3 Main zoysiagrass ancestry group cluster



Evolution of *Zoysia* in Japan



Zoysiagrass conventional breeding

Z. japonica
Variety 'Asakake'



Z. matrella
Wild Zoysia 'Zanpa 2'



X



- Broadleaves
- fast growing
- High dormancy



- Small leaf blade
- Low dormancy



Zoysiagrass molecular breeding



Embryogenic callus induced from stolon node.



1 hour

Infection of callus with *Agrobacterium*.



8° C, dark incubation, 5 day

Co-cultivation stage of infected callus.



Rinse to remove excess *Agrobacterium*.



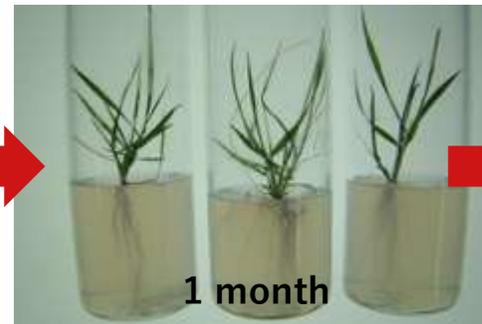
3 months

Emergence of resistant callus at selection stage.



2 months

Shoot regeneration of transformed callus.



1 month

Root regeneration.



Plants were transplanted into soil.

Development of 3D Digital Phenotyping System (DPS) for zoysiagrass

“Phenotyping bottleneck”



Full Paper
Sequencing and comparative analyses of the genomes of zoysiagrasses
 Hidemori Tanaka^{1,2}, Hideki Hirakawa^{1,2}, Shunichi Kasuga², Shinobu Nakayama², Aiko Ono², Aiko Watanabe², Masatsugu Haseguchi¹, Takahiro Gondo², Genki Ishigaki¹, Malody Mugerza², Katsuya Shimizu², Noriko Sawamura², Takayasu Inoue², Yuichi Shigeki², Naoki Ohno², Satoshi Tabata², Ryo Akashi¹, and Shusui Seto^{1,2*}

Establishing an abundant and efficient explant system for the *Agrobacterium*-mediated transformation of zoysiagrass
 J. Wang¹, Y. Hoshino¹, T. Yamada¹
¹ Graduate School of Environmental Science, Hokkaido University, Kita 10, Nishi 10, Eda, Sapporo 060-0811, Japan
² Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811, Japan, E-mail: yamada@es.hokudai.ac.jp



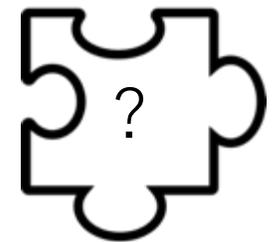
Genome

Proteome

Phenome

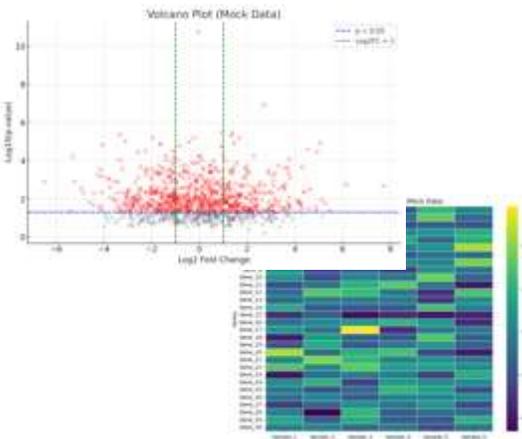
Transcriptome

Etc.



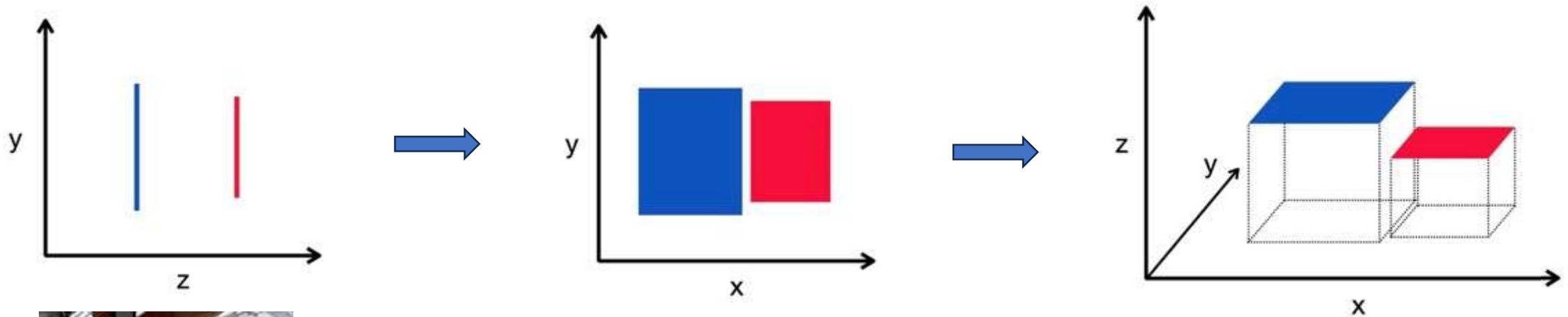
High-throughput phenotyping
 Big Data, Large-scale,
 Fast, Low-cost

Crop Science
 ORIGINAL RESEARCH ARTICLE
Differences in proteome response to cold acclimation in *Zoysia japonica* cultivars with different levels of freeze tolerance
 Jessica M. Brown, Kingsang Yu, H. McCamp P. Holloway, Michelle DeCosta, Rachel P. Bernstein, Jefferson Lu, Yan-Q. Tang, Abeer J. Fattou, Jeffrey C. Dunne, Christian Avallone ... See all authors
 First published: 23 May 2020 | <https://doi.org/10.1002/csc2.30225> | CiteSpace: 2



RESEARCH ARTICLE Open Access
Physiological and transcriptomic analyses reveal the mechanisms underlying the salt tolerance of *Zoysia japonica* Steud
 Jingjing Wang, Cong An, Hailin Guo^{*}, Xiangyang Yang, Jingbo Chen, Junqin Zeng, Jianjun Li and Jianshi Liu

Transition to 3D model phenotyping



- Destructive
- Subjective
- Time-consuming

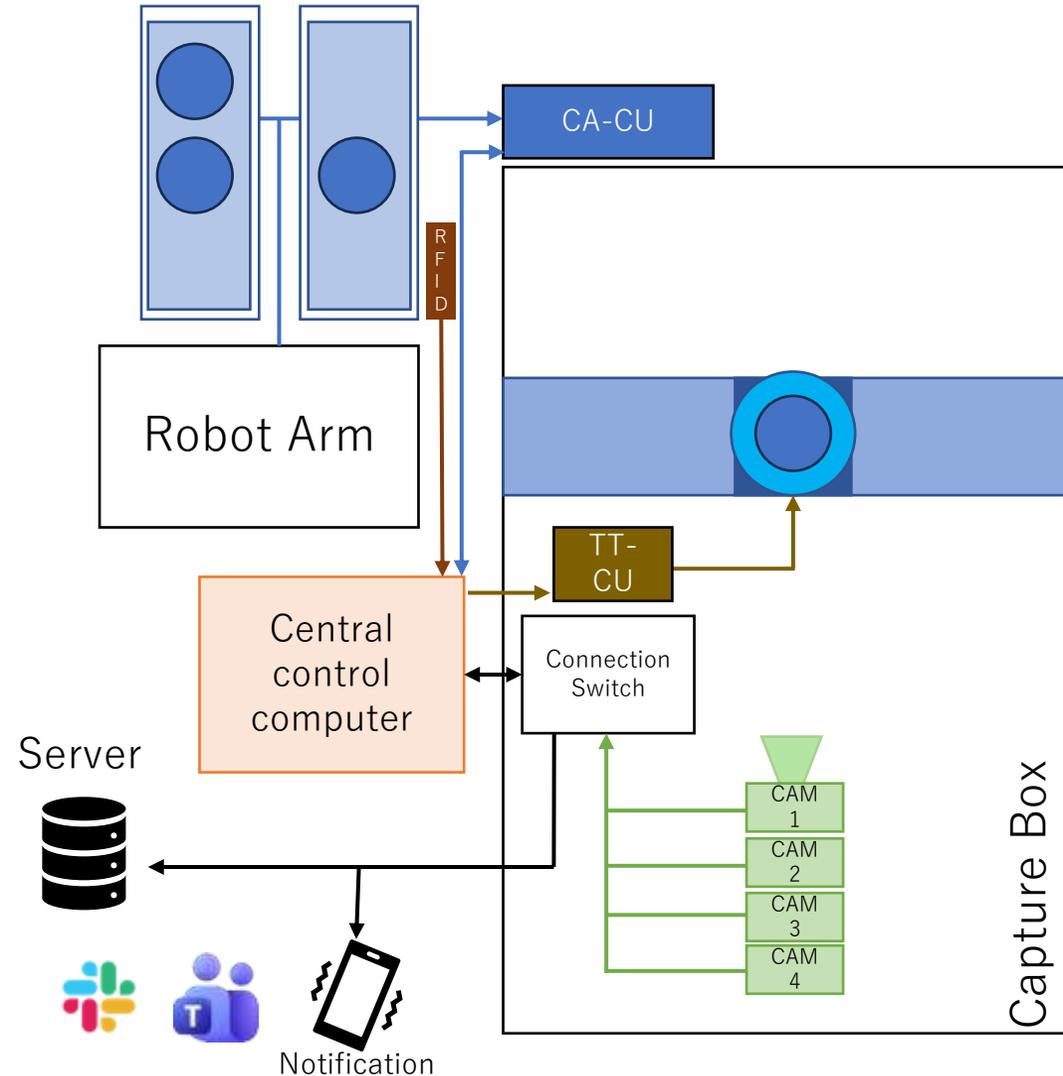


- More efficient but...
- Loses 3D structural information
- No representation of canopy volume

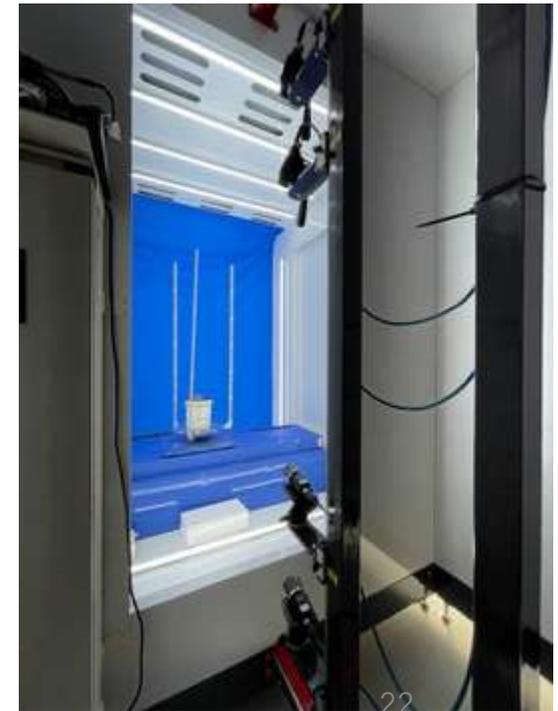


- Depict real object
- No dimension loss
- Reproducibility for data collection

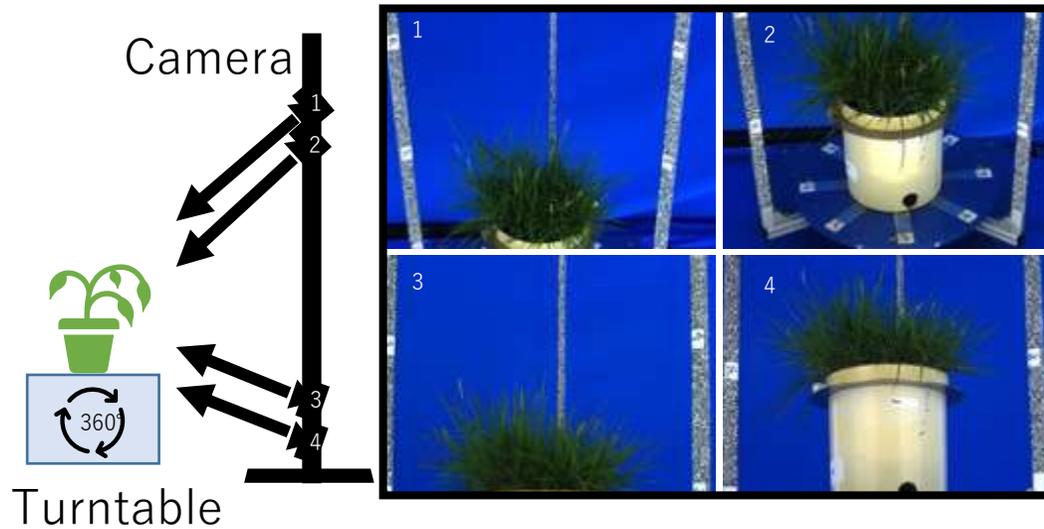
Digital Phenotyping System (DPS)



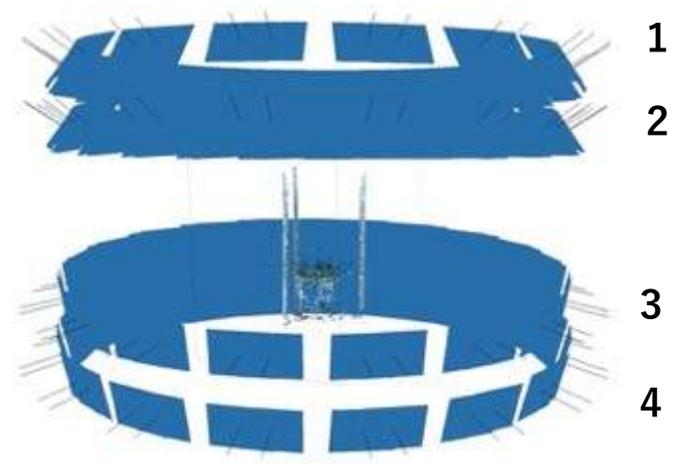
View inside capture box



3D model reconstruction



Metashape Application



Structure from Motion (SfM)

Reconstructed 3D model



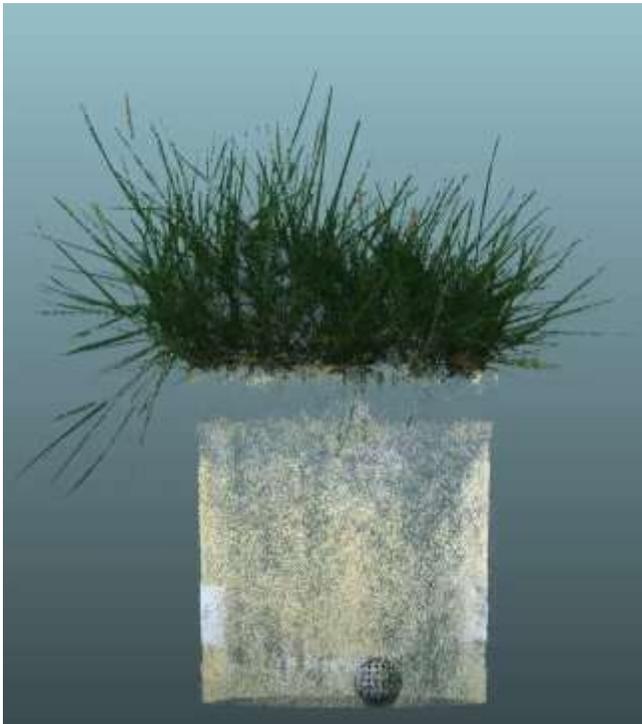
Deep learning-based segmentation



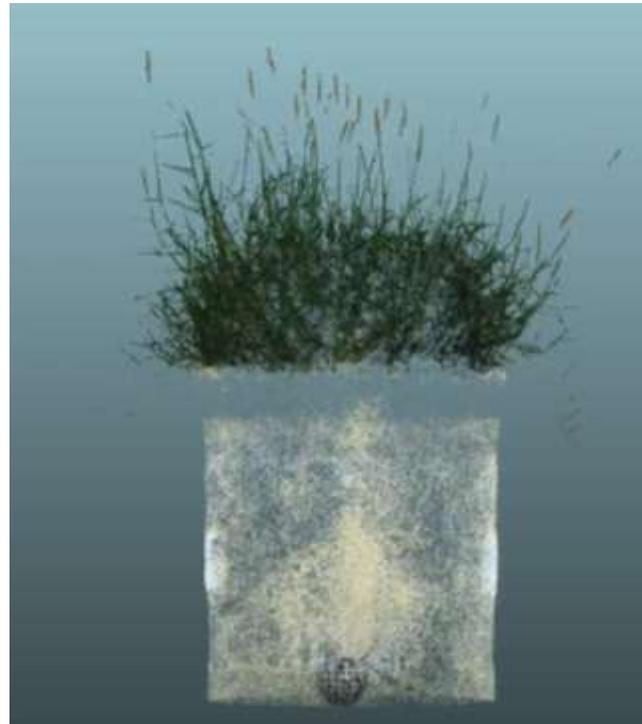
Segmented plant 3D model

Reconstructed 3D models of zoysiagrass

Z. japonica



Z. matrella

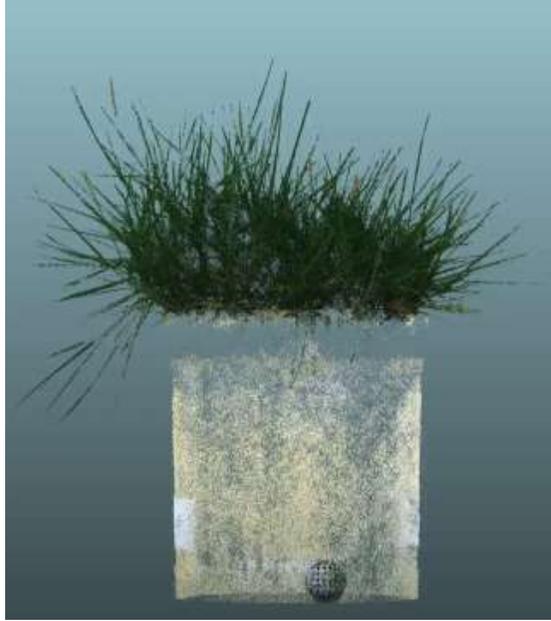


Z. pacifica

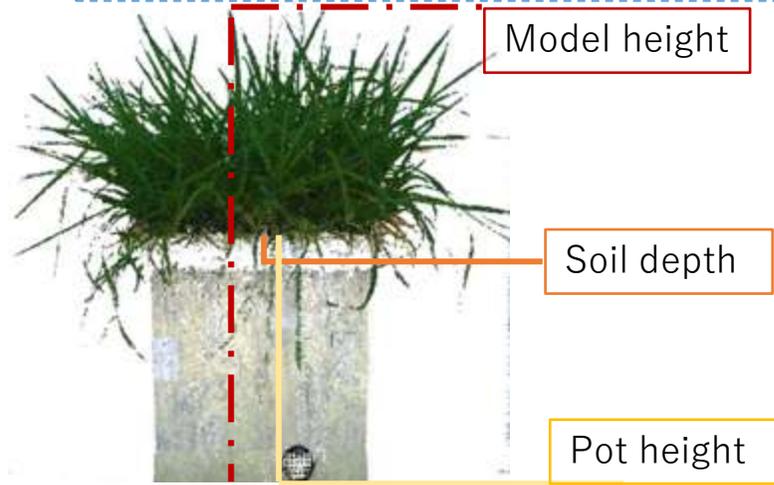


Plant morphological analysis using 3D model

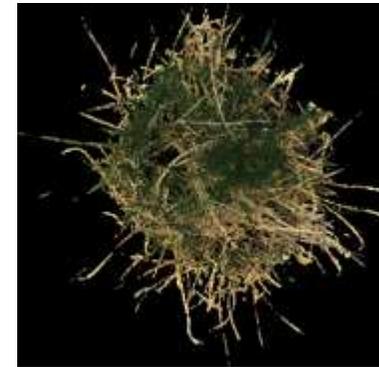
3D Point cloud model



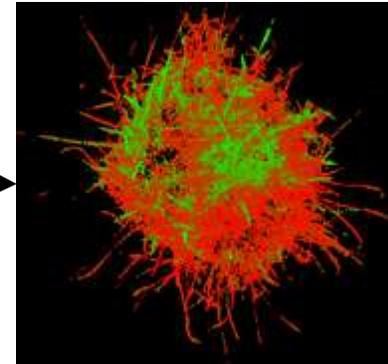
$$\text{Plant height} = \text{Model height} - (\text{Pot height} - \text{Soil depth})$$



Plant color index

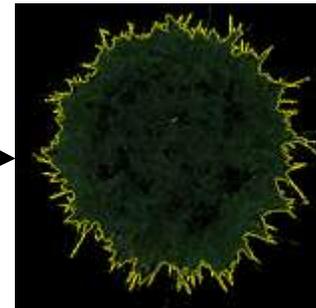
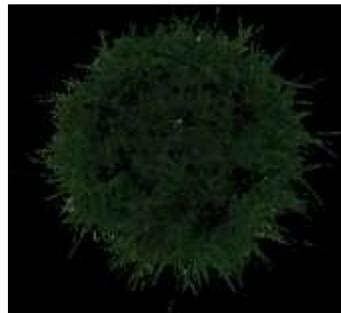


$$\text{Green-Red Normalize Difference Index (GRNDI)} = \frac{G - R}{G + R}$$



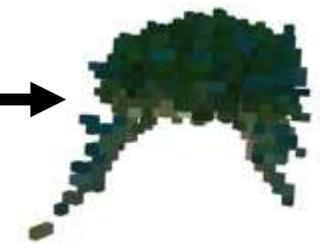
Plant Area

Contour Area



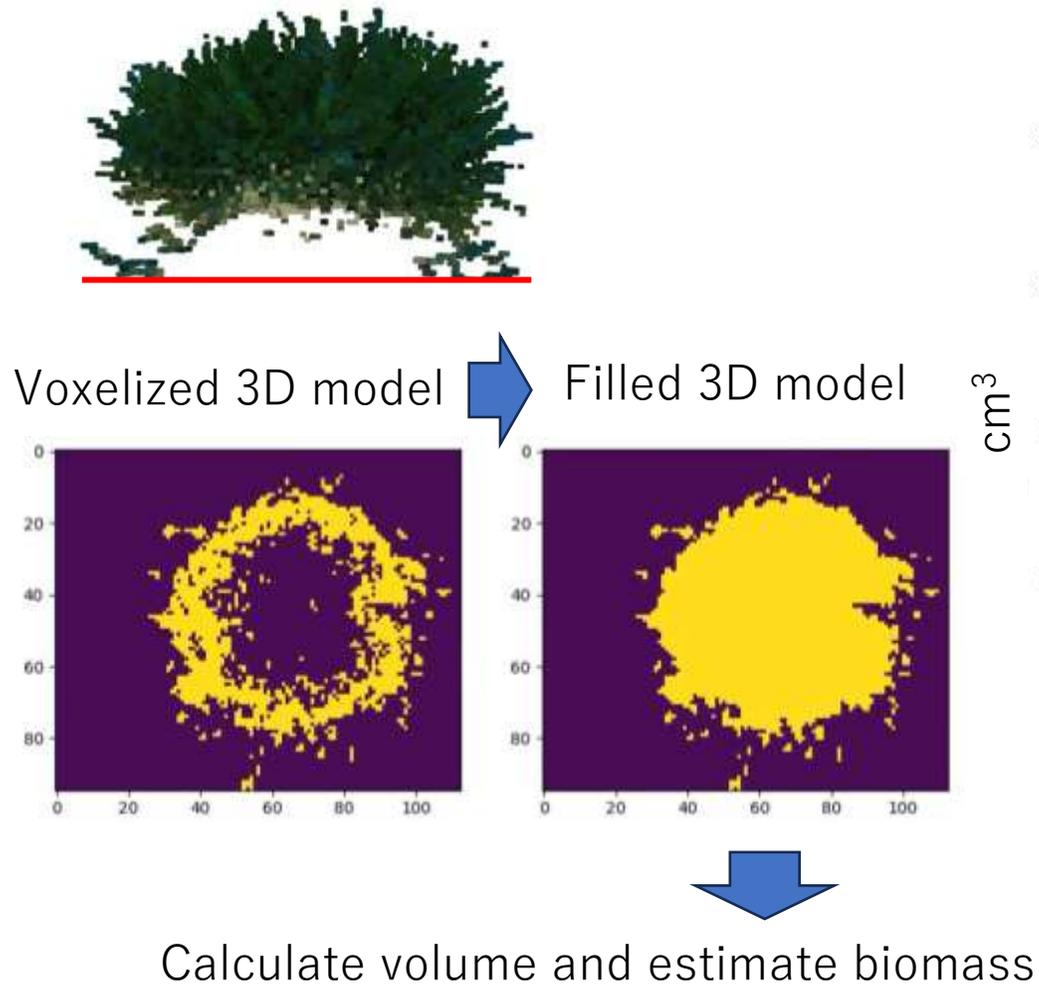
Plant Volume

Voxelization

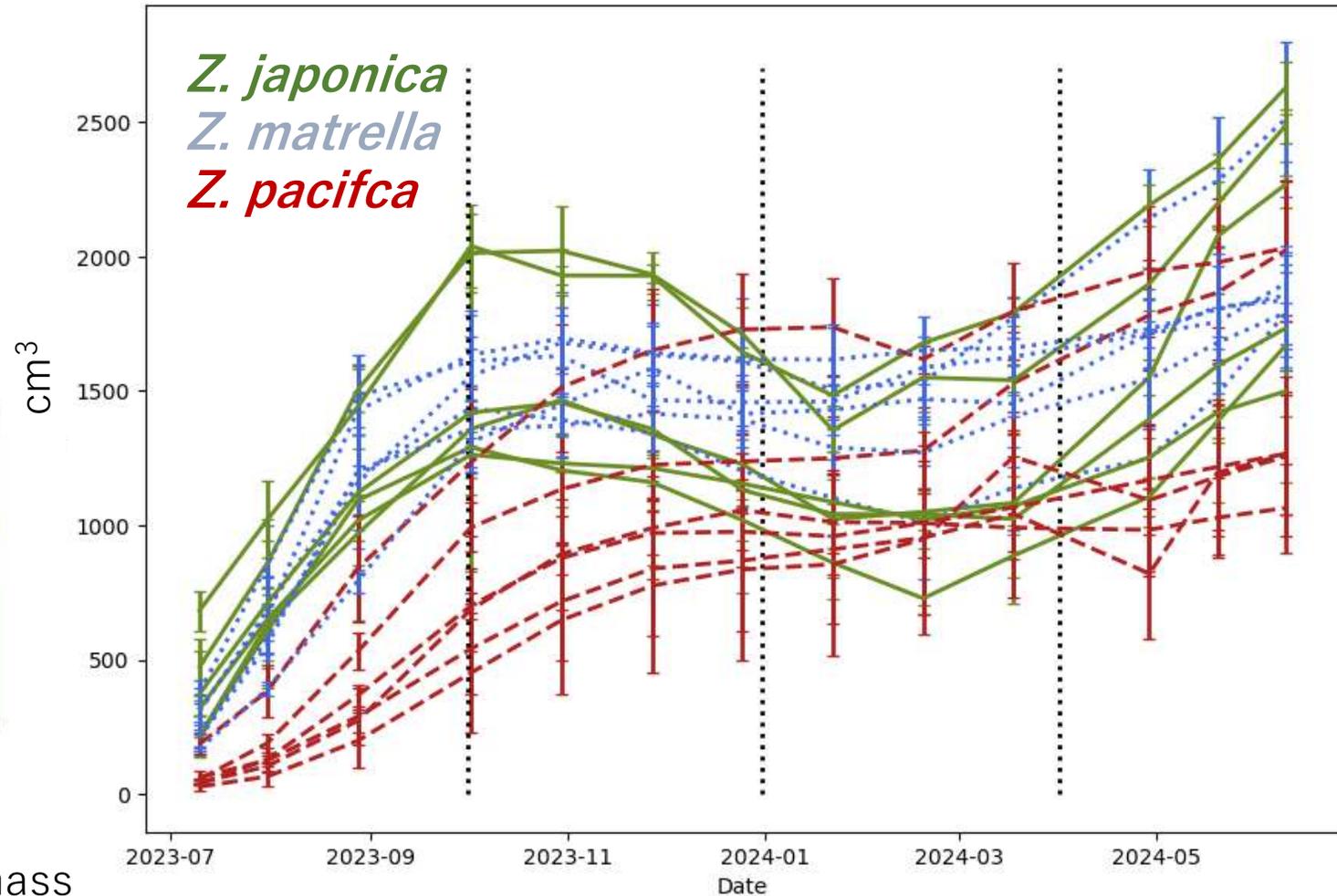


Z. japonica *Z. matrella* *Z. pacifica*

3D Phenotyping advantage: Volume



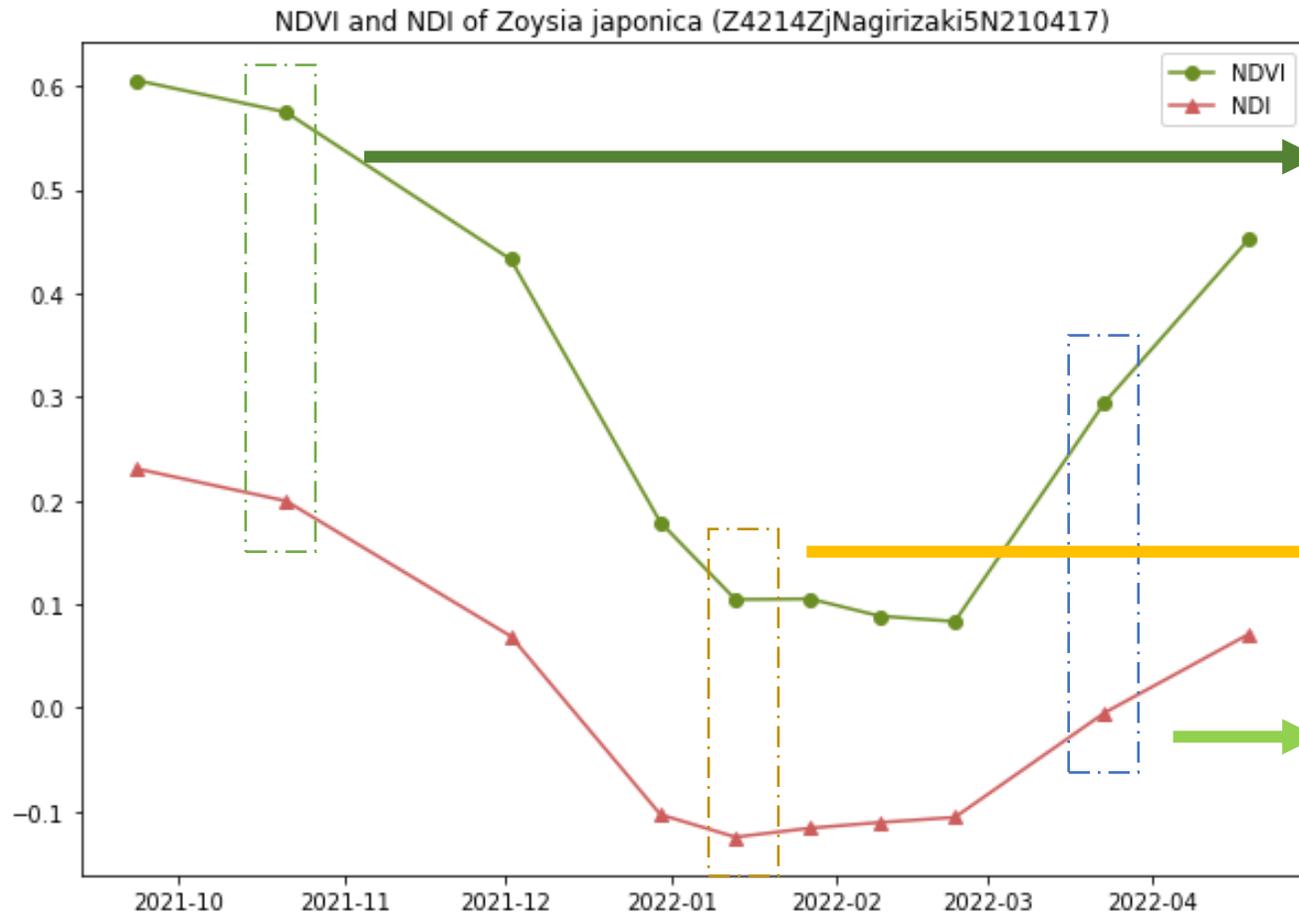
Plant biomass (Volume, cm³)



Comparison of GRNDI to NDVI



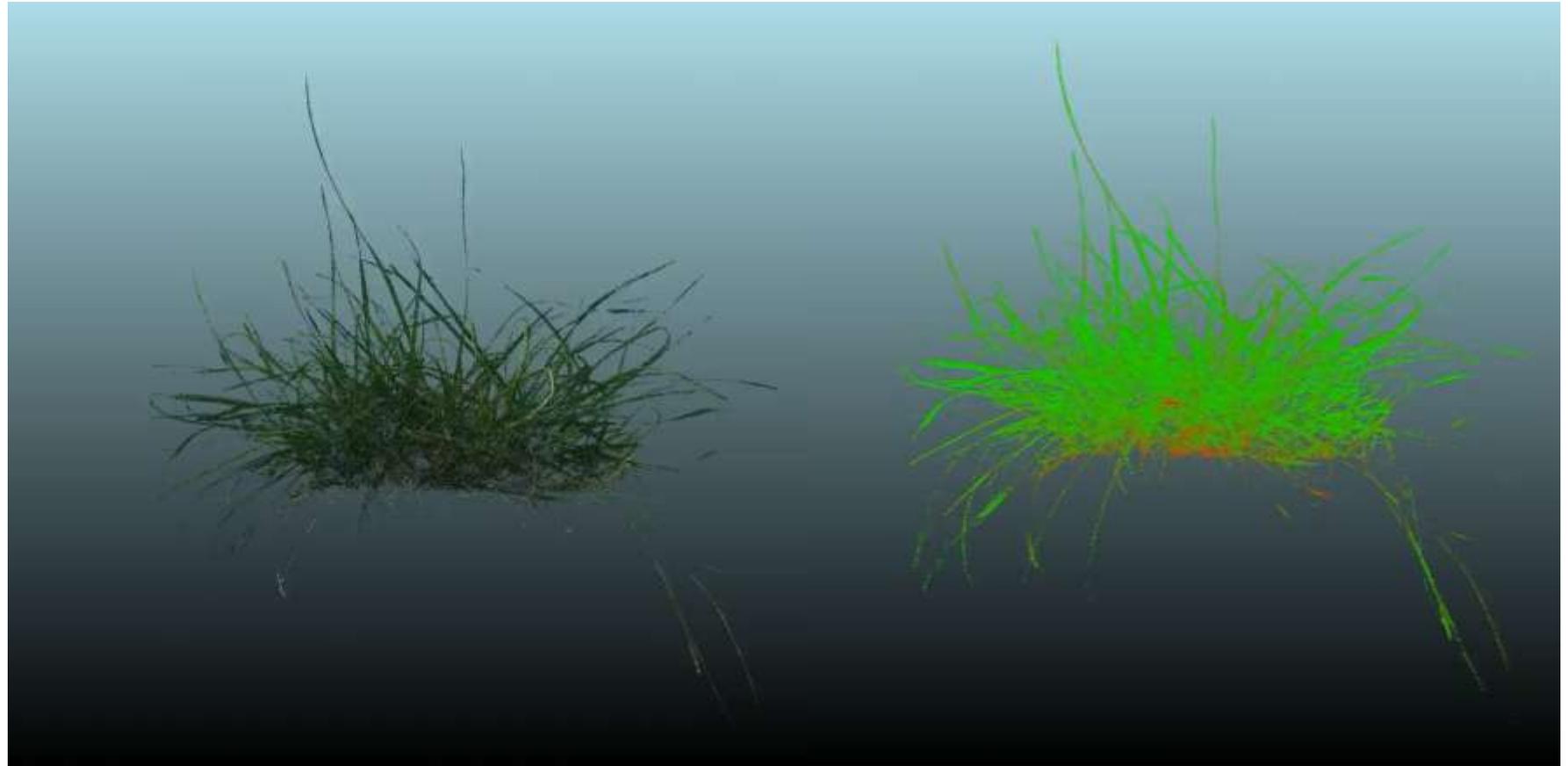
Measuring NDVI using Spectrum Technology TC500 Turf Color Meter



Color indices: visualized in 3D

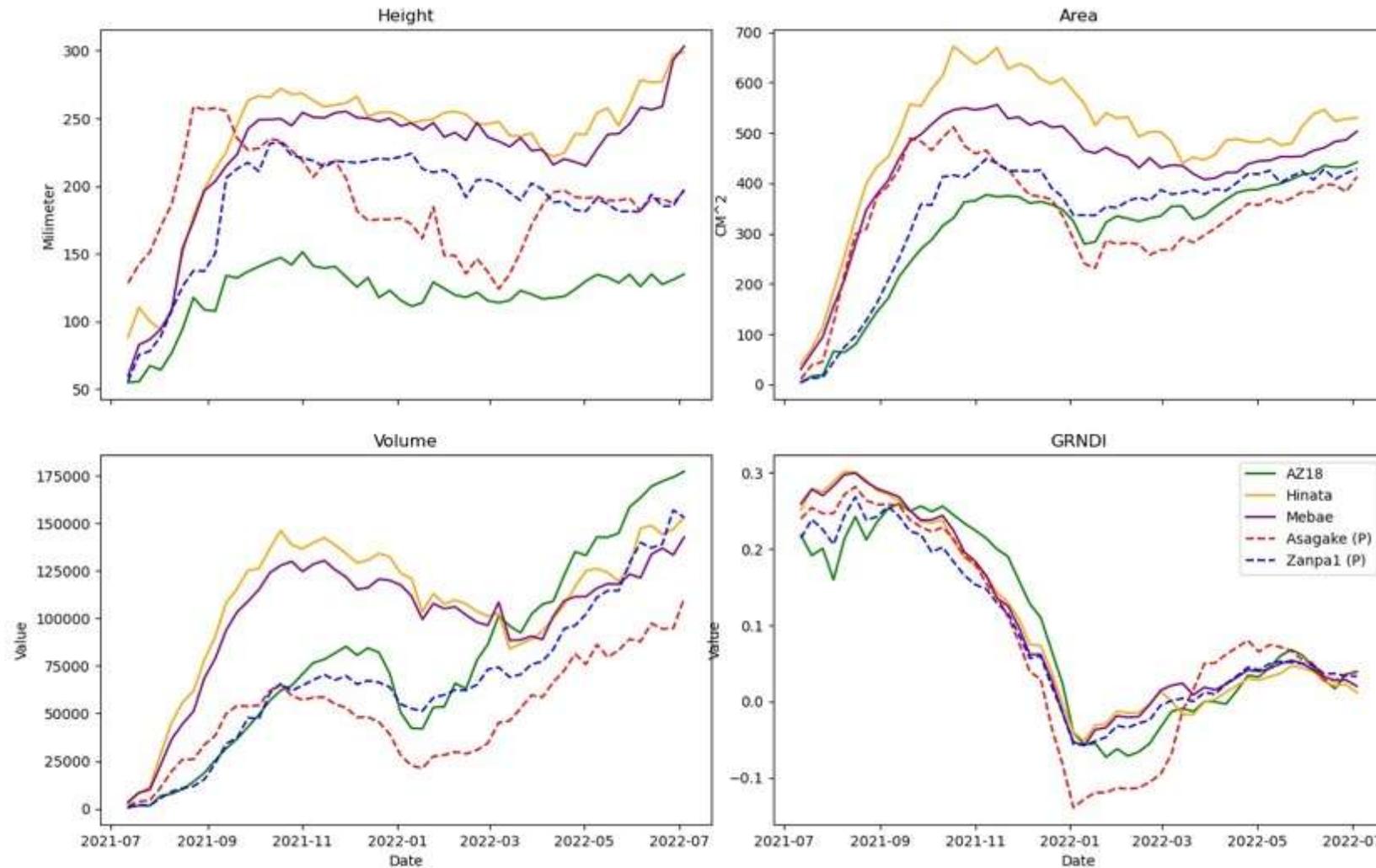
Beyond non-contact measurement

- Quantifies color across the entire plant
- Extracts phenotypic information unavailable through conventional approaches



0.4 GRNDI -0.1

Example of DPS potential in breeding program



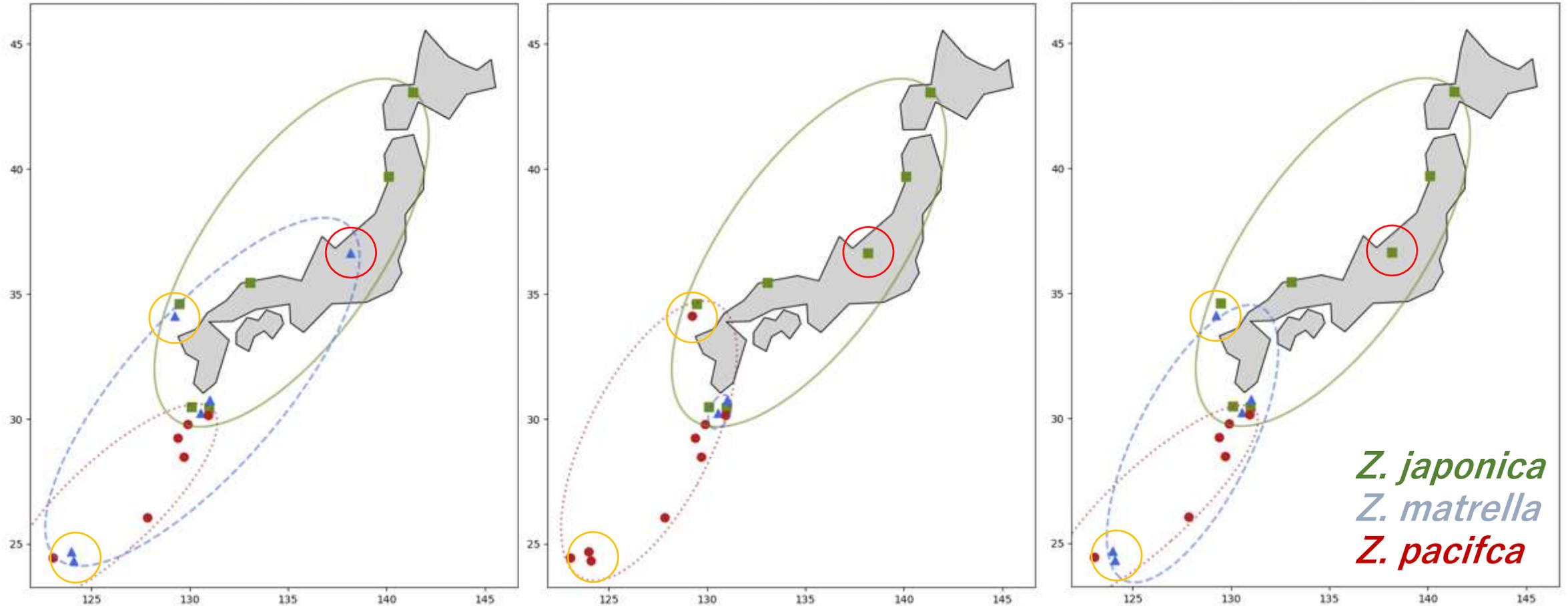
--- Parent line (A,Z)
— F₁ Line

Diversity assessment and classification of zoysiagrass ecotypes

Manual Observation

SSR Markers (Genotype)

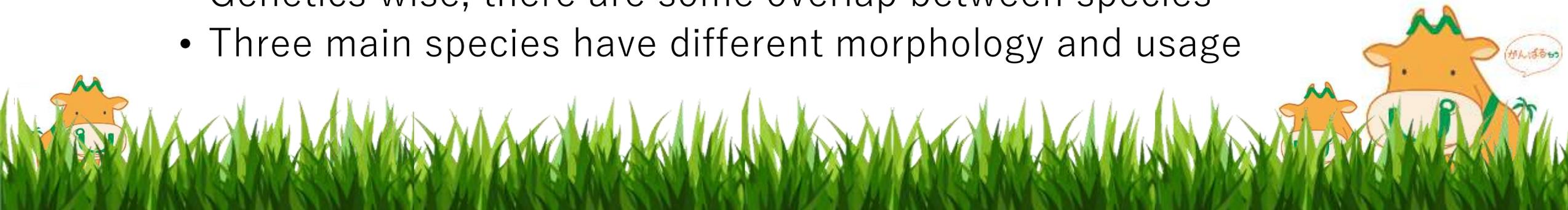
Digital Phenotype + Growth trend



Conclusion

Zoysiagrass: Key Takeaways

- Zoysiagrass is a culturally and economically important turfgrass in Japan and Asia
 - Use as lawn grass, sport turf, forage grass
- Displays substantial morphological diversity across species
 - Genetics wise, there are some overlap between species
 - Three main species have different morphology and usage



Conclusion (2)

- Traditional classification based on morphology alone is often ambiguous
 - Leaf width etc.
- Now using digital technology we can increase speed in phenotyping and breeding and give more data to look at
 - Volume, GRNDI
 - Data-driven strategy
- Combine with genomic data to further increase research output in zoysiagrass and other turfgrass



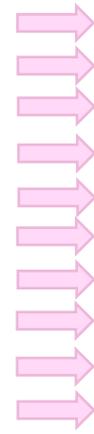
Implementing 3D-DPS into breeding research

Conventional Breeding

Plant genetic resource



Breeding methods



Plant selection

Field Trials

High cost: labour, time, money, place

→ = Candidate plant

Next-generation Breeding

Plant genetic resource



Breeding methods



Digital 3D-Phenotyping System

Field Trials

Plant selection

- Precise
- Data driven breeding
- Reduced cost
- Efficient

Acknowledgement

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- JST CREST (grant number JPMJCR1601)
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- The Ito Foundation (grant numbers 39 (2023) and 38 (2024))
- Nippon Steel Engineering Co., Ltd.

The logo for JST CREST, featuring the word "CREST" in a bold, teal, sans-serif font.

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The Ito Foundation

Reference

Pongpiyapaiboon S, Tanaka H, Hashiguchi M, Hashiguchi T, Hayashi A, Tanabata T, Isobe S, Akashi R (2023) Development of a digital phenotyping system using 3D model reconstruction for zoysiagrass. *Plant Phenome Journal* 6: e20076.



Hayashi A, Kochi N, Kodama K, Isobe S, Tanabata T (2025) CLCFM3: An advanced photogrammetry algorithm for high-precision plant 3D modeling. *bioRxiv* 10.1101/2024.10.10.617704

Tanabata T, Kodama K, Hashiguchi T, Inomata D, Tanaka H, Isobe S (2022) Development of a plant conveyance system using an AGV and a self-designed plant-handling device: A case study of DIY plant phenotyping. *Breed Sci* 72: 85–95.