Australian wild rice characterisation
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by Robert Henry

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Foreword

This project funded a PhD student for 3 years to study the genetics of Australian wild rice. The research has confirmed that native Australian rice populations are key genetic resources for rice globally. Rice breeders targeting varieties for production in areas where rice is a native plant in northern Australia will benefit from access to genetic resources adapted to the local environment including local rice diseases. The development of a wild rice industry in northern Australia will also be facilitated. Selected wild rice populations should be conserved as a genetic resource by controlling weeds and other threats to these populations.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report is an addition to RIRDC’s diverse range of over 2000 research publications. This report forms part of our New and Emerging Plant Industries R&D program, which aims to conduct research, development and extension for new, emerging and other core funded plant industries that contribute to the profitability, sustainability and productivity of regional Australia.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

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About the Author

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Warren Strevens NPARC/ Apudthama Land & Sea Ranger (Injinoo)

Ernest Madua Napranum Ranger and traditional owner

“Uncle Shorty”, Traditional Landowner, Far North Cape area
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Executive Summary

What the report is about

Wild rice populations in northern Australia may be key genetic resources for rice improvement globally. They may be of special value in supporting the breeding of conventional rice with adaptation to local Australian environments and in the establishment of a novel Australian wild rice industry. This project has characterised the genetics of these Australian populations to determine their novel value and likely utility.

Who is the report targeted at?

Rice breeders, growers, processors, and markets will be guided by this analysis of the Australian rice germplasm.

Where are the relevant industries located in Australia, what is the location of the strongest industry representation in Australia?

Rice is currently grown in southern Australia (NSW and Victoria) by more than 1000 producers. Production has exceeded 1 million tonnes per year with most being exported. Local production in southern Australia has been limited in recent years due to water availability. This has renewed interest and production in North Queensland. The global market for rice is large and growing.

Rice producers especially those in North Queensland will benefit from this research.

The demand for rice has increased in recent years (FAOSTAT, 2013). World rice production is approximately 480 million tons of milled rice in 2014/2015. Global rice demand will to need to increase by at least 80 million tons in the next twenty years to meet demand of around 560 million tons of milled rice.

Background

Rice is the major staple food crop in Asia and a major contributor to world food supply. The recent discovery of two apparently new taxa of wild rice (Oryza) in north Queensland has raised the possibility that Queensland may be not only a centre of global diversity for rice but also a possible centre of origin. Rice (Oryza sativa) was domesticated in Asia (probably China) about 7000 years ago but the new Queensland taxa are apparently ancestral to the clade (group) of species that include domesticated rice.

The newly identified taxa are key resources for rice improvement globally because they can be interbred with rice and have novel diversity that could contribute, for example, resistances to pests and diseases. These species may also have potential as novel new high value crops for production in North Queensland.

Aims/objectives

This project aimed to assessment available Australian wild rice resources, support some additional collections and genetic analysis to characterize this germplasm and define relationships to domesticated rice. The research was conducted in support of conventional rice breeding and establishment of an Australian wild rice industry.
Methods used

This project supported the analysis of rice collected in Queensland by next generation sequencing to determine the sequence of the genomes.

Results/key findings

Wild rice was collected in April and May 2014 and 2015 from Cairns north to the tip of Cape York. Samples of the main types were analysed by DNA sequencing. An *O. rufipogon* like type was found to have a nuclear genome similar to Asian wild rices (*O. rufipogon*) but a chloroplast genome that was closer to the Australian wild material (*O. meridionalis*). Another perennial (*O. meridionalis*) type had genomes that were similar to the annual *O. meridionalis*. The results clearly demonstrated that the Australian populations were distinct and were a sister group to the group containing all domesticated rices. Ongoing effort to analyse the diversity in the collected material will continue over the next few years. The evidence confirmed that the Australian wild rice populations were a diverse source of novel germplasm for global rice production. These A genome group species are inter-fertile with domesticated rice allow easy gene transfer. The results also support the value of establishing a native wild rice industry to produce this native Australian food. Conservation of these species in the wild will be enhanced by the knowledge gained in this study.

Implications for relevant stakeholders

- A taxonomic revision of the *Oryza* genus is likely with the prospect of at least one new Australian species that might be recognised as ancestral to the clade that generated domesticated rice in Asia and Africa.

- Commercial interest in developing a uniquely Australian wild rice product has been generated. Wild rice will be sold at a significant premium as evidenced by the high prices payed for wild rices from North America.

- Australian wild rice is likely to become a key genetic resource for global rice breeders. This project has generated significant public and scientific interest in Australian wild rice globally.

- Rice production in northern Australian is likely to expand because of available water, recognition that rice is native to these regions and the availability of germplasm with adaptation to northern Australian conditions, especially native Australian rice diseases.

Recommendations

- Environmental agencies should be encouraged to take steps to protect selected wild rice populations in situ. The main threats to these populations seem to be competition from weeds that are increasing at many sites.

- A taxonomic revision of the A genome *Oryza* should be completed to better support and focus conservation and utilization of this genetic resource.

- International collaboration should be used to support extensive collection of seed for ex situ conservation in Australian and international seed banks.

- The establishment of a local Australian wild rice industry should be encouraged as a companion to expansion of domesticated rice production in northern Australia.
Introduction

Rice is an important food crop globally. Sustainable production of rice requires continued genetic advances to address the challenges of climate change and the need for greater efficiency in the use of water. Wild rices growing across Northern Australia probably represent the most diverse sources of genetic variation that can be easily captured by conventional rice breeding. Australian wild rice is poorly characterized but provides a major opportunity for rice improvement in Australia and globally.

Rice was domesticated in Asia but the range of the wild species from which rice was domesticated extends to northern Australia. Australian wild populations retain pre-domestication diversity that has apparently been lost outside Australia due largely to the impact of domestication. Recent research has highlighted the international significance of this Australia genetic resource for rice improvement globally.

The genetics of wild Australian populations is just emerging and structured collections are now required to define population diversity and taxonomy. Novel qualities that may be promoted as having health benefits will be selected in this germplasm. Australian wild rice will be developed both for cultivation as a novel species and as a genetic resource for domesticated rice.
Objectives

Workshop: A workshop on Australian wild rice will be held in April 2012 to review research on this topic and to allow analysis of collections to date and define needs for further collection.

Collection: Wild rice from north Queensland populations will be collected to establish a public germplasm collection to complement and fill gaps in earlier collections. A seed collection, living plant collection and DNA collection will be developed. Existing collections often include seed or DNA bulked form more than one plant. The populations now require structured sampling to define genetic structure and taxonomic relationships. A Ph D student will be engaged to conduct this study.

Screening: The collected material will be screened for useful variation quality traits that will give a health marketing advantage.

Crossing: Selected lines will be available for crossing with Australian domesticated rice varieties to provide foundation germplasm for selection of varieties incorporating new diversity and useful traits and also to identify material of value as wild rice cultivars.
Methodology

The project evaluated collections of wild rice to date, material was collected to fill gaps and allow genetic analysis of the wild material. Next generation sequencing was used to characterize the genetics of the populations and determine their value in rice breeding. Useful traits in this material were assessed by collaboration with specialists. Populations of wild rice and cultivated rice were established for use by rice breeders.

The project has been conducted by Ph.D. students supervised by Robert Henry and several other co-supervisors. One student Marta Brozynska was funded by the project and others have developed related projects with funding from other sources.

Project Details for Marta Brozynska

“Australian wild rice”

This project sequenced the chloroplast and nuclear genomes of wild rices in Australia and determine their identities and relationships.

Project Details for Peterson Wambugu

“Genetic diversity of African rice”

This project will explore diversity in wild rices in Africa. Molecular analysis of African taxa has resolved some relationships. The control of amylose content in a cross between Asian and African rice is being used to determine the genetic basis of the high amylose content of wild and domesticated rice in Africa.

Project Details for Tiparat Tikapunya

“Grain quality of Australian wild rice”

This project targets an analysis of the grain quality of wild rice populations in Australia. Physical characteristics such as grain size and shape are being determined. Grain colour is being assessed. Starch properties are being analysed in detail and sensory evaluation of the cooked rice is proposed.

Project Details for Ali Imad Mohammad Moner

“Exploring gene diversity in the genome of wild rice populations”

This project will explore application of next generation sequencing to plant genomics and plant improvement. Specifically it will use whole genome sequencing to define genes controlling adaptation to abiotic stress including drought and salt. The genomes of wild rice populations growing in contrasting environments will be analysed to identify genetic mechanisms of adaptation to climate that could be exploited in domesticated crops. This builds on recent research showing the value of genome analysis in wild populations for the understanding of how to adapt agriculture to variable and changing environments.

Methodology will include collection of wild populations, DNA sequencing and sequence data analysis.

This project will contribute significantly to developing climate resilience in rice and other major food crops to improve global food security.

Project Details for Hayba Qassar Younan Badro
“Application of genotyping by sequencing to analysis of diversity and marker assisted selection in rice.”

New DNA sequencing technologies will be applied to the analysis of rice.

This project will employing the latest next generation sequencing tools to develop methods for diversity analysis, genotype fingerprinting and marker assisted selection in rice as a model for application in plants more generally.

Advances in DNA sequencing technology are reaching the stage where whole genome analysis may become cost effective for genotyping in major crop plants. This project will evaluate and apply these technologies to rice. International genomics resources being developed for rice will be analysed to identify alleles of value in rice selection and breeding.

Project details for Kai Wang (supervisors Robert Gilbert, Robert Henry)

“Genetics of rice starch properties”

Project Details for Wanporn Khemmuk (supervisors; Andrew Geering, Robert Henry, Roger Shivas)

“Wild rice ice pathogens in Australia”

This project is evaluating the genetics and identity of endemic rice pathogens.

Project details for Loraine Watson (supervisors still being finalized)

“The archaeological history of wild rice in Australia”

This project will investigate the recent history of sites were wild rice is found.

The key method employed in this project has been next generation sequencing (NGS) using largely Illumina platforms but a Life Technologies platform has also been compared. Chloroplast and nuclear genome sequencing has been used to define the genetic relationships and genome resources provided by the Australian wild rice populations.

Analysis of data has been supported by the University of Queensland, Research Computing Centre.
Results

This project has initiated a renewed interest in wild rice in Australia. The work of a Ph D student funded by the project, Marta Brozynska, has sequenced of the chloroplast and more recently the nuclear genomes of two important Australian taxa. The project has been the catalyst enabling new projects to be developed including an ARC funded discovery project, “Australia: The centre of origin and or the centre of diversity for rice”. International media coverage has been high, especially in the popular media with stories in Nature and Sunday California Magazine and recent interviews for New Scientist and National Geographic (Appendix). A team of 8 Ph D students has been built working on various aspects of these key global resources for rice food security. Ph D projects are examining diversity within Australian rices, grain quality of Australian rice, stress tolerance genes, genomics of the Australian taxa, taxonomy of the Australian rices, Australian rice pathogens, archaeology of rice sites, new technologies for rice genetic analysis and comparisons with diversity in African rices. Scientists form China and India have joined the project in Australia to examine diversity in the material and the stress tolerance genes that are present. Comparisons with germplasm in China and India and now proposed in projects in these countries.

The two main A genome wild taxa have been subjected to genome sequencing. Assembly of the genome is continuing and will be completed with the Ph D project in mid-2016. The full significance of these genome will only then be determined. Complete chloroplast genome sequences were obtained (Figure 2) and used to determine relationships between rice taxa (Figure 3). This clearly shows the Australian group (clade) as sister group to the Asian and African clades that include the two domesticated rice species.

Workshop: A workshop on Australian wild rice will be held in April 2012 to review research on this topic and to allow analysis of collections to date and define needs for further collection.

Workshop: A workshop on Australian wild rice was held in April 2012 to review research on this topic and to allow analysis of collections to date and define needs for further collection. Robert Henry attended an Expert Consultation Workshop on the use of crop wild relatives for pre-breeding in rice in Odaira, Shizuoka, Japan, 29th-30th October 2013. The workshop was organized and funded by the Global Crop Diversity Trust. Australia was identified as a priority area in this workshop and this project will align with their global objectives for the capture of rice genetic resources for food security. A white paper “Diversity Seek (DivSeek) Harnessing the power of crop diversity to feed the future” was prepared with an international group of scientist with support of the Global trust following a workshop in San Diego in January 2015.

Collection: Wild rice from north Queensland populations will be collected to establish a public germplasm collection to complement and fill gaps in earlier collections. A seed collection, living plant collection and DNA collection will be developed. Existing collections often include seed or DNA bulked form more than one plant. The populations now require structured sampling to define genetic structure and taxonomic relationships. A Ph D student will be engaged to conduct this study.

A major collection (Figure 1) and observation trip in North Queensland was held on 12-14 May 2014. Participants included QAAFI staff (Robert Henry and others) and students (Marta Brozynska, Peterson Wambugu, Tiparat Tikapunya) and Ian Chivers (Native Seeds). Permits were obtained from the Queensland Government (Queensland Parks and Wildlife Service), Land Trust Traditional Owners of Rinyirru National Park and Mareeba Shire Council.
Figure 1 Collection team in Mareeba at wild *Oryza* installation by Japanese artist at Mareeba wetlands
Figure 2 Chloroplast genome sequence of *O. rufipogon* like taxa form north Queensland (Brozynska et al., 2014)
Figure 3 Relationships between wild rices based upon chloroplast genome sequences (Wambugu et al., 2015)
A second major collection of plant tissue and seed from across Cape York (from Cairns to the tip of Cape York) was conducted in May 2015 in a period of two weeks collecting between Cairns and the tip of Cape York. A total of 250 samples are currently being analysed to define the distribution of diversity within these Australian wild rice populations. DNA has been extracted from all of these collections. This should provide a platform for enhanced conservation and utilization of this important genetic resource.

Plants in the glasshouse at St Lucia and Toowoomba have been established in separate areas to avoid cross pollination between potentially different taxa.

Samples of the main types were analysed by DNA sequencing. An O. rufipogon like type (Figure 4) was found to have a nuclear genome similar to Asian wild rices (O. rufipogon) but a chloroplast genome that was closer to the Australian wild material (O. meridionalis). Another perennial (O. meridionalis) type (Figure 5) had genomes that were similar to the annual O. meridionalis. The results clearly demonstrated that the Australian populations were distinct and were a sister group to the group containing all domesticated rices. Ongoing effort to analyse the diversity in the collected material will continue over the next few years. The evidence confirmed that the Australian wild rice populations were a diverse source of novel germplasm for global rice production. These A genome group species are inter-fertile with domesticated rice allow easy gene transfer. The results also support the value of establishing a native wild rice industry to produce this native Australian food. Conservation of these species in the wild will be enhanced by the knowledge gained in this study.

Figure 4 Wild rice (taxa A) in north Queensland
Marta Brozynska was engaged as the Ph D student on the project. Many other Ph D students have enrolled in related and supporting projects with funding from a wide range of other sources.

Screening: The collected material will be screened for useful variation quality traits that will give a health marketing advantage.

Plants have been observed in the glasshouse in Brisbane and Toowoomba. Extensive analysis of seed physical characteristics of wild collected material showed that seeds well of a similar length to domesticated rices but generally more slender. Grain characteristics under cultivation with good nutrition need to be established. Starch properties are currently being studied in detail and should be completed by mid-2016. Sensory evaluation studies will also be conducted on cooked wild material when sufficient quantities of rice are available in 2016. Parallel work on African rice has identified the high amylose content of *O. glabberima* as an important characteristic. We have shown that this trait can be traced to the wild progenitor species (*O. bathii*). A population of a cross between African and Asian rice has been imported from Africa and is being used to determine the genetic basis of this trait. Similar studies should eventually be possible with Australian wild rices.
Figure 6 Wild rice in the glasshouse at St Lucia showing the shattering trait

Crossing: Selected lines will be crossed with Australian domesticated rice varieties to provide foundation germplasm for selection of varieties incorporating new diversity and useful traits and also to identify material of value as wild rice cultivars.

Dr Ryuji Ishikawa has provided extensive data on the success of crossing Australian taxa collected several years ago with domesticated rice. This has confirmed that these populations are inter-fertile with domesticated rice. Significant differences in the numbers of successful pollination events were obtained reflecting differences in genetic distances between the populations. Further selection of wild germplasm will be required to determine the most appropriate sources to use in rice breeding. Progress towards use in breeding will also require a clarification of intellectual property issues and establishment of benefit sharing agreements where necessary. A cross between Africa and Asian rice as described above is being used to explore approaches for eth capture of useful genetic variation from these wild populations.

Results to date have been published in the following journal manuscripts:


Related Publications


**Key conference reports**


Henry RJ (2015) Improving food and energy security by applying biochemical and molecular tools to the development of improved crop varieties. Seed Business 2015 Convention Toowoomba


Henry RJ (2015) Genome sequencing to support germplasm analysis and utilization. 5th International Conference on Next Generation Genomics and Integrated Breeding for Crop Improvement, Hyderabad


Henry RJ (2014) Research and education to address food, water, energy, environment and climate vulnerabilities. McDonnell Symposium St Louis


Henry RJ (2014) Innovation in agriculture in the tropics: the Australian perspective Global Agri-Food Forum Mexico


Henry RJ (2014) Plant Barcoding by NGS analysis of chloroplast genomes in total plant DNA samples. Plant Genomics USA St Louis


Henry RJ (2013) Genomics for cereal nutrition and quality in a changing climate Agricultural Biotechnology International Conference Calgary

Henry RJ (2013) Functional and nutritional traits from wild rices. 7th International Rice Genetics Symposium, Manila.


Krishnan, SG, Waters DLE, Henry RJ (2012) Genome-wide variations between elite lines of indica rice discovered through whole genome re-sequencing. International Symposium on "100 years of rice science and looking beyond" Coimbatore
Implications

Assessment of the impact of the outcomes on industry in Australia (where possible provide a statement of costs and benefits)

Implications

-A taxonomic revision of the Oryza genus is likely with the prospect of at least one new Australian species that might be recognised as ancestral to the clade that generated domesticated rice in Asia and Africa.

-Commercial interest in developing a uniquely Australian wild rice product has been generated.

Wild rice will be sold at a significant premium as evidenced by the high prices payed for wild rices from North America. The Queensland wild rice will have the added selling point of being an ancestor of domesticated Asian rice unlike the North American wild rice. In this sense the Queensland product may be marketable as the ‘ONLY TRUE WILD RICE’. The size of the market in Asia for this product is likely to be significant. This will generate local employment in rice production and processing in regional north Queensland. Several regions could benefit, including the Burdekin, Tully (wet tropics), Atherton Tablelands and the Gulf. Retail prices for wild rices in Queensland are currently $30-50 per Kg while conventional domesticated rices are in the range of $1-4 per Kg. These prices ensure that even low yielding first generation domesticates of the wild rice will be able to be produced profitably. The market for rice of all types in Asia is very large and this new product could expect to find a large market as a novelty food in Queensland and also worldwide, especially in Asia. This industry will generate significant employment opportunities in regional areas in north Queensland along the value chain form on farm production to processing and retail sales.

-Australian wild rice is likely to become a key genetic resource for global rice breeders.

-Rice production in northern Australian is likely to expand because of available water, recognition that rice is native to these regions and the availability of germplasm with adaptation to northern Australian conditions, especially native Australian rice diseases.
Recommendations

- Environmental agencies should be encouraged to take steps to protect selected wild rice populations in situ. The main threats to these populations seem to be competition from weeds that are increasing at many sites.

- A taxonomic revision of the A genome Oryza should be completed to better support and focus conservation and utilization of this genetic resource.

- International collaboration should be used to support extensive collection of seed for ex situ conservation in Australian and international seed banks.

- The establishment of a local Australian wild rice industry should be encouraged as a companion to expansion of domesticated rice production in northern Australia.
Appendices

Press and media coverage

1. Food Navigator-Asia.com: Queensland wild rice could save crop if supply were to be compromised June 11 2014
   [http://www.foodnavigator-asia.com/Policy/Queensland-wild-rice-could-save-crop-if-supply-were-to-be-compromised](http://www.foodnavigator-asia.com/Policy/Queensland-wild-rice-could-save-crop-if-supply-were-to-be-compromised)

2. ABC Rural: Northern Australia ‘vision’ less likely without research focus, May 15 2014


4. ABC Bush Telegraph: Australian wild rice could hold key to global food security, July 9 2014

• Radio Interview on ABC Queensland 2015

- Blue Sky Rice, article appeared in Nature, October 2014

See PDF attachment

- Sunday California Magazine


- New Scientist

On Tuesday 15th September, journalist Richard Schiffman interviewed Robert Henry for an article on Australian Wild Rice to be published in New Scientist Magazine.

Summary of recent contributions from Henry research group on rice quality

Fragrance in rice

The availability of a rice genome sequence allowed rapid identification of the gene for fragrance in basmati and jasmine rice (Bradbury et al., 2005a). This development has greatly enhanced the breeding of fragrant rices by providing perfect molecular markers for use in screening (Bradbury et
The gene, annotated as betaine aldehyde dehydrogenase (Fitzgerald et al., 2009) acts on 4-aminobutaldehyde (Bradbury et al., 2008). The identification of the biochemical pathway leading to fragrance has revealed that this trait is linked to a loss of abiotic stress tolerance (Fitzgerald et al., 2008; Fitzgerald et al., 2010).

**Starch properties of rice**

The starch properties of rice are key to many of the quality attributes of this high starch grain that is consumed as a whole grain product. Functional SNP associated with control of the gelatinization temperature of rice starch were identified (Waters et al., 2006). Amplicon sequencing strategies (Henry et al., 2012) were used to evaluate the role of variation in starch biosynthesis genes in the control of starch properties in the Australian rice breeding germplasm pool (Kharabian-Masouleh, 2011; Kharabian-Masouleh, 2012). This identified those genes that showed variation that was associated with differences in starch properties in the gene pool. Patents on starch manipulation in plants have been reviewed (Waters and Henry, 2007).

**Markers for rice quality**

High through-put genetic marker assay tools for use in selection for rice quality and other attributes were developed to support rice breeding (Kharabian-Masouleh, 2009). Re-sequencing techniques for whole genome SNP discovery for marker applications (Edwards and Henry, 2011) were evaluated in Indica rices (Gopala Krishnan, 2012). A protocol for SNP extraction from whole genome next generation sequencing data of rice was documented (Krishnan et al., 2014).

**Tools for transgenic manipulation of rice quality**

Promoters for the control of gene expression in the grains of rice were tested in rice (Furtado et al., 2008) and other species.

**Quality of wild rice relatives**

Crop wild relatives are an important source of genetic diversity for use in crop improvement. Advances in genome sequencing technology (Henry, 2013) have made the genomes of wild relatives more accessible for gene discovery.

The starch properties of the grains of wild Oryza species do not show the diversity of properties that have been selected in the domesticated gene pool (Kasem et al., 2013). However they do show great variation in whole grain colour and form (Kasem et al., 2010) but less variation in endosperm morphology (Kasem et al., 2011). The sequences of starch biosynthesis genes were related in accordance with the known relationships between the species (Kasem et al., 2012).

Many areas especially in Africa (Wambugu et al., 2013) and Australia (Henry et al., 2010) are poorly explored and may contain novel wild rices with useful genes for rice improvement. Recent molecular analysis has revealed novel populations of close relatives of domesticated rice in northern Australia (Sotowa et al., 2013). Simple whole chloroplast genome sequencing methods have been developed and applied to analysis of relationships between wild rice populations (Nock et al, 2011). These approaches have demonstrated the distinctness of the Australian germplasm (Waters et al., 2012) and clearly distinguished Australian taxa that appear morphologically similar to Oryza rufipogon (Brozynska et al., 2013).
More distant relatives of rice may have potential for rapid domestication as novel crops for new production environments (Shapter et al., 2013). Genomes sequencing may be used to accelerate domestication (Henry, 2012) by providing direct access to the sequences of domestication genes (Malory et al., 2011) that can be targeted for mutation.

Study of genome variation in wild rice relatives has been proposed as a guide as to how to adapt crops to climate.
References


Molecular relationships between Australian annual wild rice, Oryza meridionalis, and two related perennial forms. Rice, 6, 26.


Other publications not on rice but relevant.


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