

# Breeding of ‘Manten-Kirari’, a non-bitter and trace-rutinosidase variety in Tartary buckwheat, and *de novo* sequencing of transcriptome and mass discovery of SNPs

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## ABSTRACT

‘Manten-Kirari’, a new Tartary buckwheat (*Fagopyrum tataricum*) variety has developed. *In vitro* rutinosidase activities of ‘Manten-Kirari’ was three orders magnitude less than that of rutinosidase-normal variety such as ‘Hokkai T8’. In ‘Hokkai T8’, rutin in dough was completely hydrolyzed within one hour after addition of water. On the other hand, most part of rutin was present without hydrolysis in ‘Manten-Kirari’ dough. In ‘Hokkai T8’ flour, most panellists feel strong bitterness whereas no panellists feel bitterness in ‘Manten-Kirari’. From these results, ‘Manten-Kirari’ would be promising as material for rutin-rich/ non-bitterness foods. To develop SNPs-based DNA marker for variety discrimination, we performed *de novo* sequencing of transcriptome in seedling including roots using GS-FLX+ Titanium and HiSeq 200. Using these data, we tried mass discovery of SNPs between ‘Manten-Kirari’ and ‘Hokkai T10’, an EMS treated mutant of ‘Hokkai T8’. Although 81 % of SNPs detected by ‘VarScan’ software was excluded as false-positives, we employed residual 19 % as ‘tentative SNPs’.

## INTRODUCTION

Rutin, a kind of flavonoid, exhibits beneficial effects on human health (Wieslander et al., 2011). Tartary buckwheat is known to be a useful food because it contained about 100 times higher amount of rutin in its seed than that of common buckwheat. However, Tartary buckwheat seed also contains huge amount of rutinosidase activity than common buckwheat (Yasuda et al. 1994; Suzuki et al., 2002). The rutinosidase activity in Tartary buckwheat seeds was sufficient to hydrolyze rutin in the flour within a few minutes after the addition of water. Therefore, variety with trace-rutinosidase activity has been required. In addition, Tartary buckwheat is traditionally known as ‘bitter buckwheat’. The foods containing Tartary buckwheat flour sometimes have strong bitterness. Therefore, it has not widely spread into the diet in Japan. From these backgrounds, we developed a new Tartary buckwheat variety ‘Manten-Kirari’. Here, we show its breeding procedure and traits of the variety about bitterness and rutin hydrolysis. In buckwheat, *de novo* sequencing using next generation sequencing (NGS) about florals (Vinogradov et al., 2011, Yasui et al., 2012) and developing seeds (Chen et al., 2012) have been reported. However, to date, *de novo* sequencing in seedling including root have not yet reported. Therefore, we tried to obtain seedling transcriptome including root in Tartary buckwheat. We also tried to discover variant such as SNPs using transcriptomic data got from Illumina HiSeq 2000.

## MATERIALS AND METHODS

To measure *in vitro* rutinosidase activity and rutinosidase isozymes, we employed HPLC and in-gel detection method (Suzuki et al., 2002, 2004). Brief procedure of ‘Manten-Kirari’ development is

shown in Fig. 1. To investigate bitterness of flour, a sensory analysis was performed. Brief procedure and experimental design of NGS analysis are summarized in Fig. 2.

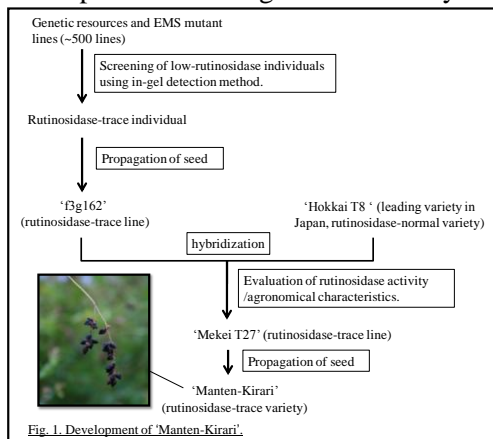


Fig. 1. Development of 'Manten-Kirari'.

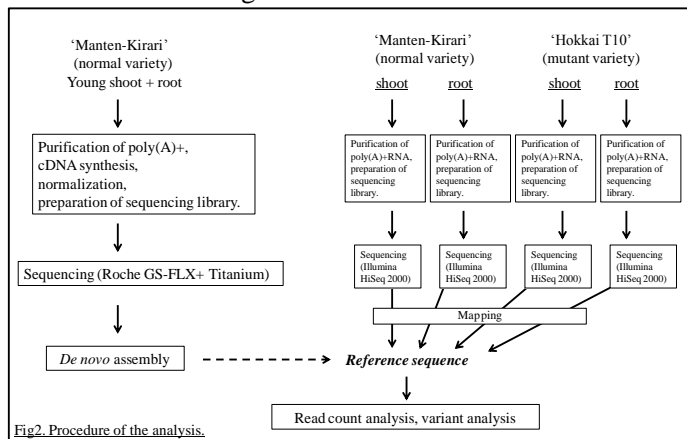


Fig2. Procedure of the analysis.

## RESULTS AND DISCUSSION

We could not detect rutinosidase isozymes in in-gel detection method both in 'Manten-Kirari' and its pod parent 'f3g162' (Fig. 3). *In vitro* rutinosidase activities of 'Manten-Kirari' and 'f3g162' were three orders magnitude less than that of

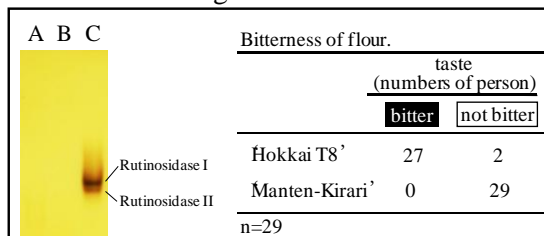


Fig. 3. Detection of rutinosidase isozymes in 'Manten-Kirari' and its parent. A: 'f3g162' (rutinosidase trace mutant; pod parent), B: 'Manten-Kirari', C: 'Hokkai T8' (rutinosidase normal variety; pollen parent). Rutinosidase isozymes in seed were detected using in-gel detection method followed after extraction and native-PAGE of crude protein.

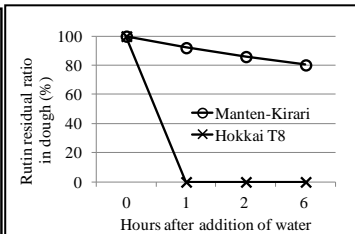


Fig. 4. Comparison of rutin hydrolysis in dough between 'Manten-Kirari' and 'Hokkai T8'. Water (45ml per 100g flour) was added to the flour, and then sufficiently mixed. The dough was stored at 5°C for 1, 2 and 6 hours. Rutin was extracted and measured using HPLC. Rutin residual ratio was expressed relative concentration against 0 hour (just after addition of water).

rutinosidase-normal variety such as 'Hokkai T8'; this rutinosidase-trace characteristic was dominated by a single recessive gene (data not shown). In 'Hokkai T8', rutin in dough was completely hydrolyzed within

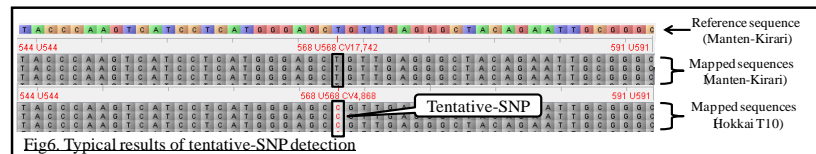


Fig6. Typical results of tentative-SNP detection

'Manten-Kirari' flour had no bitterness (Fig.3). Hand-made buckwheat noodle made from 'Manten-Kirari' flour also had no bitterness whereas 'Hokkai T8' had strong bitterness (data not shown). From these results, 'Manten-Kirari' would be promising as a material for rutin-rich/ non-bitterness foods. We obtained 256Mb sequence in GS-FLX+ Titanium sequences. After MIRA assembler analysis, we obtained 11,358 large contigs (1 kb<, 7.1 reads<). In Illumina HiSeq 200 sequencing, we obtained 17.67Gb data. Mean Q of these sequencing were above 35. We considered that sequencings were performed appropriately. Generally, 'VarScan detected SNPs' were present in both 'Hokkai T10' and 'Manten-Kirari' at the same nucleotide position (Fig.5). Therefore, we exclude these SNPs as false-positive SNPs (by this analysis, we excluded about 81 % of 'VarScan detected SNPs', and employed residual 19 % as 'tentative SNPs').

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